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Factors Influencing Differential Growth Of Rat Mammary Tumor Fragments And Cells Transplanted in Gland-free and Gland-containing Mammary Fat Pads*

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Abstract—*Transplants from eight primary tumors induced by 7,12-dimethylbenz-(a)anthracene were placed into gland-free and gland-containing inguinal mammary fat pads of syngeneic female hosts. Samples were transplanted as* 1 mm^3 *fragments or as cell suspensions of enzymatically dissociated tumors averaging* 1.5 $\times 10^5$ cells/ml. *Similar differential growth patterns resulted from both types of transplants. In samples from two of eight tumors fragments produced more tumors than did dissociated transplants, regardless of fat pad condition. In one case dissociated transplants gave rise to more tumors than did fragments. Samples from two of eight tumors produced no differences in tumor growth from fragments and dissociated transplants. Samples from the remaining two showed low transplantability. Overall, no significant differences in tumor growth could be attributed to transplant treatment or fat pad condition. Gland-containing fat pads inhibited ductal outgrowth following transplantation while gland-free fat pads favored it. From the data, the greater the lability of the primary tumor, the greater the influence of the host microenvironment on the outgrowth potential of the tumor.*

INTRODUCTION

NUMEROUS reviews have appeared in the literature regarding tumor heterogeneity [1-4]. Much of the original work used methods to isolate the individual tumor cell lines or clones and to characterize them according to their biological properties *in vivo* and *in vitro* [5-9].

Random fragment transplantation studies of 7,12-dimethylbenz(a)anthracene (DMBA)-induced tumors reveal variable outgrowth potential *in vivo* such as ductal outgrowths and hyperplastic alveolar nodules, as well as tumors in gland-free mammary fat pads [10]. Similar variations in outgrowth potential have been demonstrated using cultured pregnancy-dependent mouse mammary tumors [11] and *in vitro* transformed mammary cells [12] transplanted into mammary fat pads *in vivo*.

Variability may arise because tumor fragments may not be representative of the whole tumor [5]. On the other hand, tumor cell suspensions, whether as a subline or from the whole tumor, make it difficult to assess how cell dissociation and/or isolation affect tumor behavior. The presence or absence of the mammary gland parenchyma may favor or inhibit the outgrowth potential [10, 13, 14].

Not many studies have been designed to examine the outgrowth potential of random fragments as compared to cell suspensions or the influence of the mammary parenchyma on outgrowth development. Accordingly, the major purpose of this study was two-fold. The first was to examine whether differential growth capabilities of cell suspensions of primary mammary tumors, presumably a homogeneous mixture of cells, will produce more consistent results than tumor fragments after transplantation. The

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second was to determine the effect of the mammary gland parenchyma on the growth of both fragment and enzyme-dissociated transplants.

The results indicated that growth capabilities of both types of transplants seemingly varied according to the overall lability of the respective tumor, which refers to its phenotypic diversity. The greater the lability, the greater the influence of the host microenvironment on the growth outcome of the tumor transplants.

MATERIALS AND METHODS

Animals

Two sublines of Lewis strain rats, inbred by brother-sister matings, were used for this study. The original breeding pairs were obtained in 1976 from the National Institutes of Health, Bethesda, MD, and from Simonsen's Laboratories, Gilroy, CA. Animals were maintained at constant temperature (27°C) with a controlled light cycle. Food and water were administered *ad libitum*.

Induction of mammary tumors

Females were fed 20 mg of DMBA (Sigma Chemicals, St. Louis, MO) dissolved in sesame oil. The carcinogen was administered by gastric intubation in two 10-mg feedings, once when the rats were 45–50 days old, and again 1 week later. Tumors developed beginning at 7 weeks after feeding. These were designated as primary tumors to distinguish them from those tumors derived from transplants.

Hosts

The hosts used for transplantation studies were syngeneic virgin females from 9 to 12 weeks of age and one group aged 22–27 weeks. Approximately one-half of them had the fourth, fifth and sixth pairs of their mammary anlagen and lymph nodes surgically removed by cauterization at 20–22 days [10]. This procedure provided gland-free mammary fat pads to be used as transplantation sites.

Transplantation of mammary tumors

Eight randomly selected primary tumors (1.5–1.8 cm diameter), each from a different rat, were excised under sterile conditions. Transplantation was done according to the method of Rivera [10]. Unless otherwise indicated, the eight tumors were designated by capital letters. Tumors A–G were transplanted into 9- to 12-week nulliparous hosts, and tumor H into 22- to 27-week hosts. Corresponding samples of all the tumors were fixed in Carnoy's solution for histological evaluation.

Preparation of cells for transplantation

Cell preparations were made by a modification of the method of DeOme *et al.* [17]. Minced tumors were incubated in 0.1% collagenase and hyaluronidase (Sigma Chemicals, St. Louis, MO) in medium 199 in a 37°C water bath and hand-shaken every 10 min until the suspension was cloudy. The suspensions were placed in disposable 50-ml centrifuge tubes and centrifuged for 10 min at 1600 rpm. Subsequent centrifugations were done in the same manner. The pellet was resuspended in 1.25% pronase (45,000 PUK/g Calbiochem, Los Angeles, CA) in medium 199 for 5 min. Washings were done in cold medium 199 at 4°C. Suspensions were filtered twice through 10 μm Nitex cut to fit a 22-mm Millipore Swinnex attached to a 20-ml syringe held in ice. Trypan blue staining indicated 80% epithelial cell viability. Cells were resuspended in medium 199 to a final concentration average 1.5×10^7 cells/ml.

Injection procedure

Using a Hamilton syringe No. 705, 10 μl of the cell suspension were injected into the right and left gland-free or gland-containing inguinal mammary fat pads.

Evaluation of latency and growth potential

Ten days after transplantation, and every 4 days thereafter, the mammary fat pads were palpated for tumors. Tumor latency was determined to be the number of days that elapsed between transplantation and initial palpability of the tumor. Tumors were allowed to grow up to 2.5 cm in diameter before the experiments were terminated. Tumor development was considered positive if palpable prior to termination. Samples of all tumors were processed for histological examination. If there were no palpable growths, animals were terminated between the 15th and 16th weeks after transplantation and the fat pads removed. Fat pads were spread on filter paper and fixed in buffered 10% formalin. After fixation, papers were removed and the fat pads were processed for whole mount examination according to the method of Rivera *et al.* [18].

Statistics

Mean latencies were compared using the Mann-Whitney *U* non-parametric statistic. Percentages were compared using arc-sin percentage transformations.

RESULTS

Differential growth of randomly selected primary tumor transplants: fragment and dissociated

Overall recovery of both types of transplants refers to all local takes, tumors, ductal outgrowths

and tumor-ductal combinations. Fragment transplants showed significantly higher takes than the dissociated transplants in the presence of the gland. There was no significant difference in the absence of the mammary gland parenchyma (Tables 1 and 2). When considering the dissociated transplants, recovery in the gland-containing fat pad was significantly lower when compared to either the non-dissociated counterpart or to the gland-free group.

Local takes were those transplants confined to the site of transplantation which did not give rise

Table 1. Differential growth capabilities of fragment and dissociated tumor transplants in gland-containing fat pads

		Tumor designation								
		A	B	C	D	E	F	G	H	Total
Fragment	Local takes	4	6	6		1			3	20
	Outgrowths									
	Palpable tumors		4	2	10	7	7	7	1	38
	Tumors and outgrowths	1					1			2
	Ductal/HAN							1		1
	Other		1							1
	Totals									
	Takes/samples transplanted	5/10	11/14	8/12	10/10	8/10	8/8	8/10	4/10	62/87
	(%)	(50)	(79)	(67)	(100)	(80)	(100)	(80)	(40)	(73)
Dissociated	Local takes		1	2		1	1	2		7
	Outgrowths									
	Palpable tumors		8		10			5		23
	Tumors and outgrowths						1	2	2	5
	Ductal/HAN									0
	Other		1							1
	Totals									
	Takes/samples transplanted	0/12	10/14	2/14	10/10	1/12	2/10	9/10	2/10	36/92
	(%)	(0)	(71)	(14)	(100)	(8)	(20)	(90)	(20)	(40)

Table 2. Differential growth capabilities of fragment and dissociated tumor transplants in gland-free fat pads

		Tumor designation								
		A	B	C	D	E	F	G	H	Total
Fragment	Local takes	2	1	1		2	2	5		13
	Outgrowths									
	Palpable tumors				10	6	8	2	1	27
	Tumors and outgrowths					2		1	2	5
	Ductal/HAN		8	4					1	13
	Other	1								1
	Totals									
	Takes/samples transplanted	3/12	9/14	5/14	10/10	10/10	10/10	8/10	4/6	59/86
	(%)	(25)	(64)	(86)	(100)	(100)	(100)	(80)	(67)	(67)
Dissociated	Local takes			2		1	1	1		5
	Outgrowths									
	Palpable tumors		12		12		3	5		32
	Tumors and outgrowths							1	4	5
	Ductal/HAN	2		2			1	1	2	8
	Other	3	1			1	1			6
	Totals									
	Takes/samples transplanted	5/12	13/14	4/14	12/12	2/12	6/10	8/10	6/8	56/92
	(%)	(42)	(93)	(29)	(100)	(17)	(60)	(80)	(75)	(61)

to palpable tumors or mammary outgrowths. There was no significant difference when fragment transplants were placed in either the intact or the gland-free fat pad. Localized fragment transplants were slightly better than the recovery of the dissociated transplant in the intact fat pad. The lowest localized recovery occurred when dissociated transplants were placed in the gland-free fat pad.

The primary tumors used as sources for the transplants varied in their ability to give rise to palpable tumors. The results of samples from two of the eight tumors (E and F) showed that fragments gave rise to significantly more palpable tumors than did their cellular counterparts irrespective of the condition of the fat pad. However, with samples from tumor B, the dissociated transplants gave rise to more tumors than did the fragments. The two extremes in growth behavior occurred with tumor D, whose transplants gave rise to 100% tumors irrespective of the type of transplant or condition of the fat pad, and with tumors A and C, from which fragment transplants gave rise to only 5 and 14% tumors respectively, whereas dissociated transplants developed no tumors at all (Tables 1 and 2).

Potential of tumor fragments and dissociated transplants to form palpable tumors in intact and gland-free fat pads

With regard to fragment transplants, samples from two of the eight tumors (B and G) grew to palpable size in the intact fat pad 29 and 70% respectively, whereas only 0 and 30% of the dissociated samples grew in the gland-free fat pad. Samples from three of the nine (D, E, F) grew equally well in both types of fat pads (70–100%). Samples from three of the nine (A, C, H) grew poorly regardless of the type of fat pad (<20%).

With regard to dissociated transplants, samples from three of eight tumors (A, C, E) failed to produce tumors irrespective of the fat pad condition. On the other hand, transplants derived from tumors B, D and G showed the same growth capability in both the gland-free and the intact fat pads. With tumors F and H transplants there was a significant increase in tumor development in the gland-free fat pad than in the intact fat pads.

Considering tumors separately from other outgrowths, the interesting finding was that when the total percentages of all tumors that grew were considered, there were no significant differences among the groups with respect to the type of transplant or the condition of the fat pad.

Other types of outgrowths were ducts that appeared normal and hyperplastic alveolar nodules (HANs). The gland-free fat pad favored ductal proliferation (Table 3). Generally, ductal outgrowths or HANs were not observed in the absence of tumors when fragment or dissociated transplants were placed in gland-containing fat pads. In one instance only, a fragment from tumor G gave rise to a small outgrowth (Fig. 1). Both dissociated and fragment transplants gave rise to combinations of tumors plus ductal/HAN combinations (Figs 2 and 3). When only ducts or HANs formed variability was observed (Figs 4 and 5).

Table 3. Comparison of specific outgrowth development from both dissociated and fragment transplants in both gland-free and gland-containing fat pads

Type of outgrowth		Fragment transplants	Dissociated transplants
Local	+ gland	32	19[c]
	- gland	22	9[a]
		c	c
Tumor only	+ gland	61	64[c]
	- gland	46	57[c]
		c	c
Tumor + outgrowth	+ gland	3	14[c]
	- gland	9	9[c]
		c	c
Ductal/HAN	+ gland	2	0[c]
	- gland	22	14[c]
		b	b
Other	+ gland	2	3[c]
	- gland	2	11[b]
		c	b

Nos represent the percentage of those recovered. All statistics were done by angular transformation using arc-sin percentage.
[a]Significant at $P < 0.05$.
[b]Significant at $P \leq 0.02$.
[c]Not significant.

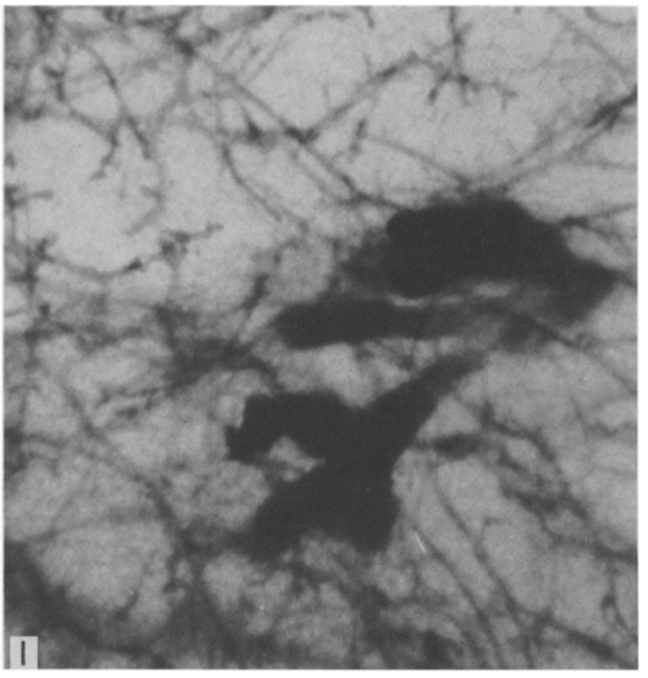
In general, the mean latencies were longer for the dissociated transplants over the fragment transplants regardless of fat pad condition.

Morphological variations of tumors were not affected by type of transplant or fat pad condition

All of the primary tumors used had features common to adenocarcinomas [19]. Common morphological patterns plus variations were noted in tumors arising from both types of transplants. Tumor C was the only one whose transplants consistently produced tumors in a similar pattern observed in the parent tumor (Figs 6 and 7).

DISCUSSION

In this study differential growth capabilities of enzyme-dissociated and fragment transplants from primary rat mammary tumor were examined. The underlying assumption was that by using enzyme-dissociated cell suspensions, greater control in cell content and cell number



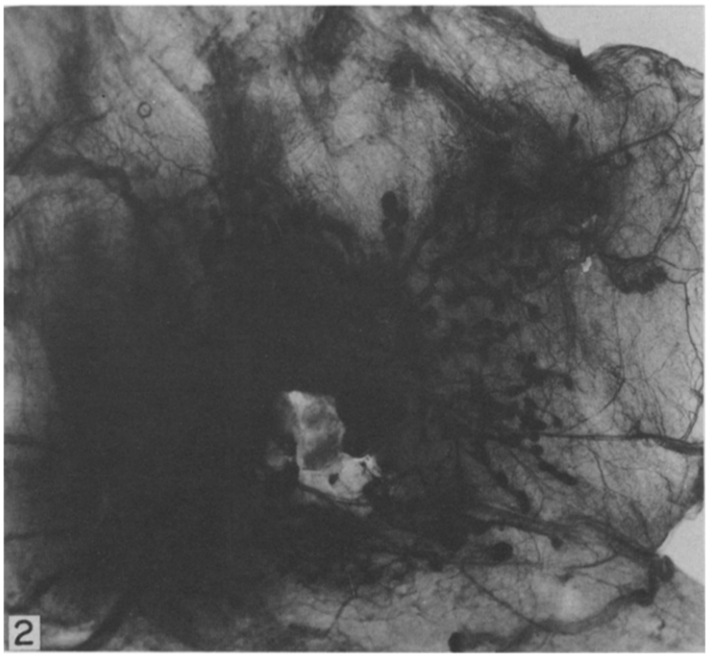
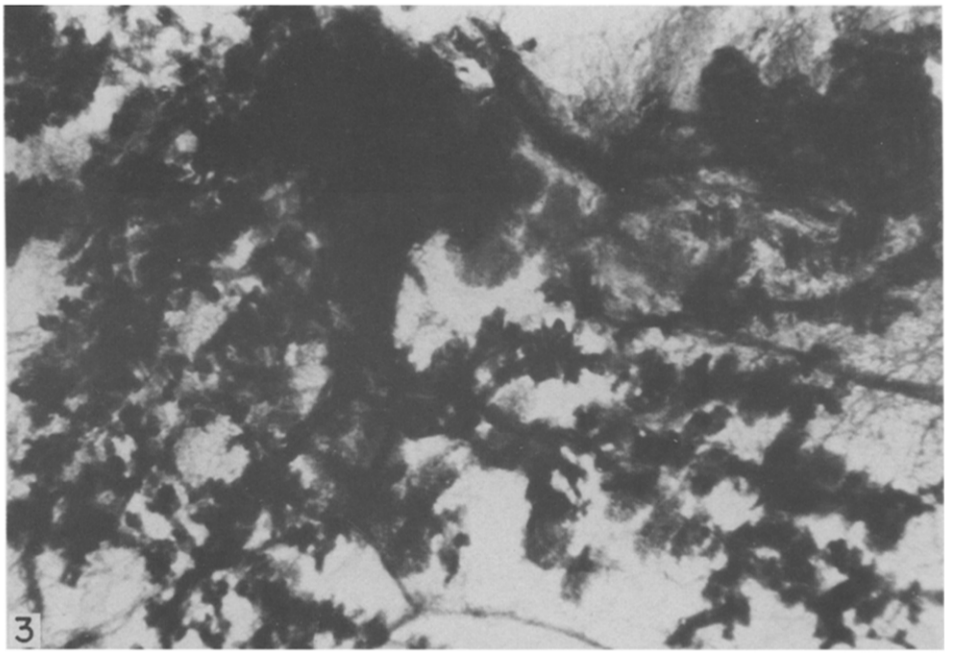
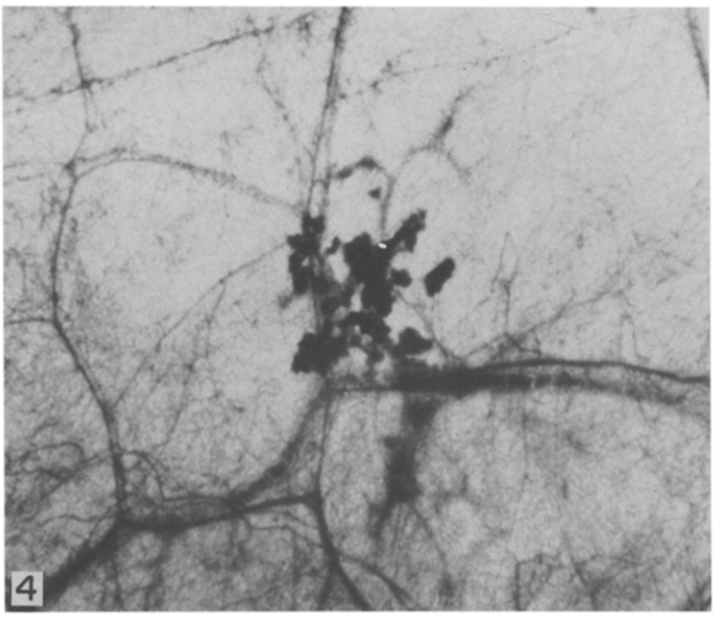


Fig. 1. Fragment from primary tumor G placed into a gland-containing fat pad. Note minimal outgrowth. ×20.

Fig. 2. Fragment from primary tumor C placed into a gland-free fat pad. Note alveolar and ductal outgrowth. Blank space indicates the site of tumor removal. ×4.





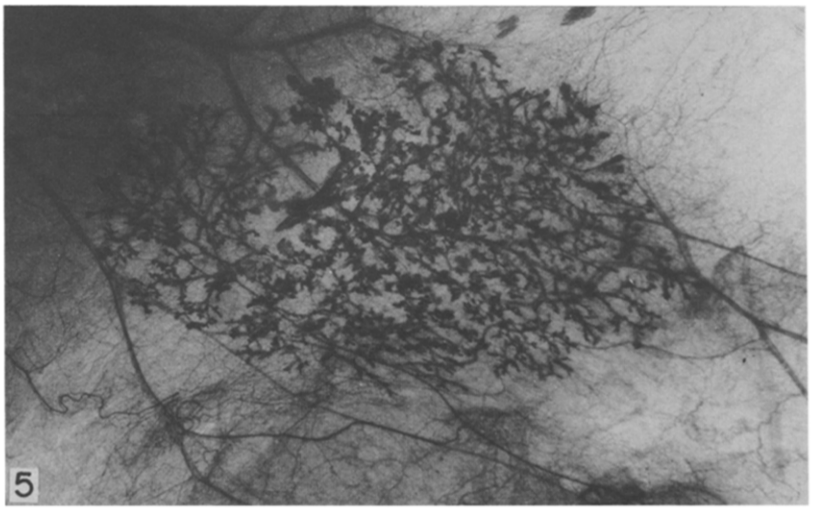
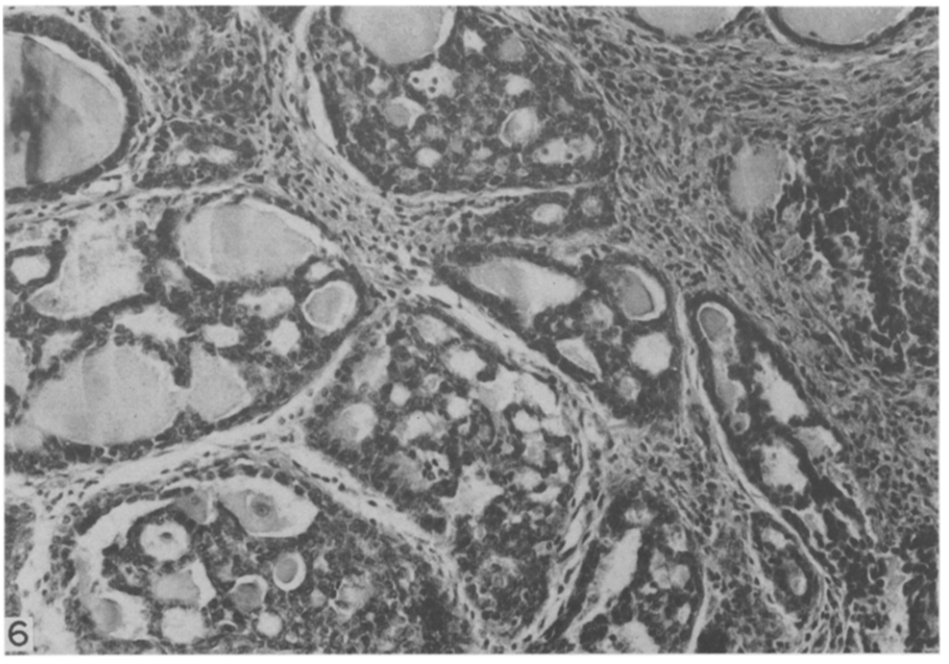


Fig. 3. Cells from enzyme-dissociated tumor H placed into a gland-free fat pad giving rise to tumor and alveolar and ductal outgrowths. ×4.

Fig. 4. Large ductal outgrowth filling the fat pad resulting from a fragment of a primary tumor B placed into a gland-free fat pad. ×4.

Fig. 5. Extensive HAN outgrowth resulting from cells of enzyme-dissociated tumor H placed into a gland-free fat pad. ×10.



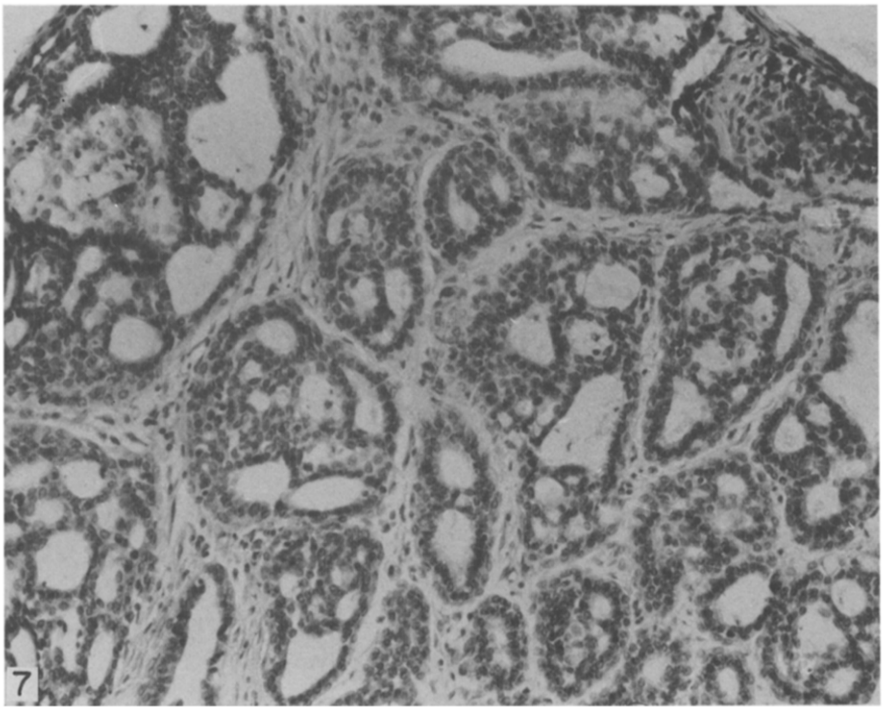


Fig. 6. A random tissue sample taken from primary tumor C. ×200.
Fig. 7. Fragment from tumor C placed into a gland-containing fat pad. ×200.

could be achieved as well as removing the cell-cell contact since cell lines tend to be regionally zoned and sister cells appear to be contiguous in solid tumors [5, 6].

The overall recovery included all local takes, tumors, tumor-ductal combinations and other ductal dysplasias. There was minimal recovery when cell suspensions were placed into gland-containing fat pads. A possible explanation is that the extensive ductal development in the gland-containing fat pad obscured the cells unless aggregation or outgrowth development occurred. There were no concordant findings of inhibition or enhancement of tumor development that was due to enzyme dissociation as shown in a previous report [20].

Both enzyme-dissociated transplants and fragment transplants were assumed to be polyclonal and gave rise to tumors, ductal dysplasias and hyperplastic alveolar nodules. These outgrowths seemed to occur independently within the same animal. Hager *et al.* [21] have suggested that the relative mixtures of subpopulations determine the subsequent expression. These investigators found fluctuations in clinical and immunological parameters after having serially transplanted sublines from mouse mammary tumors. Accordingly, subpopulations with similar characteristics might be selected out for expression at any given time. Interactions among subpopulations is an important factor in tumor behavior. Studies have been done showing that cell suspensions of tumor subpopulations react differently if injected bilaterally into syngeneic hosts alone than if in combination with another subpopulation [15, 22]. Differences were observed in incidence, latency or growth rates, depending on the subpopulations used. These investigators also showed that sensitivity of some mouse mammary tumor subpopulations to antineoplastic agents differ when tested alone or tested in the presence of other subpopulations [23]. Because of these findings, a bilateral effect in this study cannot be completely discounted.

Another critical factor in the variability of tumor expression is the host microenvironment. The host milieu can affect metastasis, growth rates and toxic agent sensitivities [24-27]. Accordingly, selective processes for these biological actions are dependent on both tumor cell properties and properties of specific organs. A similar finding was reported by Hager *et al.* using a cell line of a mouse mammary tumor that produced variants [28]. From the results of this study, the presence or absence of the mammary gland did in fact influence the behavior of some transplants. Gland-free fat pads favored ductal and hyperplastic alveolar nodule growth over the fat pads containing mammary parenchyma. It is possible that the potential for tumor cells to undergo differentiation is triggered by the gland-free fat pads [10]. To the contrary, there was a suppression of ductal/HAN outgrowth development of fragment and cellular transplants by the gland-containing fat pad. In the intact animal both fragments and cells had a tendency to remain localized if no tumor formed. It has been suggested that this may have been caused by a regulatory or inhibitory effect of normal glands on the transplants [29, 30].

These data further show that tumor development was not suppressed by the gland-containing fat pad and are in agreement with other studies demonstrating that highly tumorigenic cell populations override the inhibitory effects of normal glands [29-31]. Thus it seems apparent that the ability to produce tumors is due to intrinsic factors of the primary tumor.

Not only was there no evidence of autonomous tumor suppression, there was also no evidence of enhancement of tumor development by the intact fat pad in all cases. It should be noted that fragment samples from two tumors (B and G) out of eight did grow better in intact than in gland-free fat pads. Thus the environmental conditions provided by the host had variable influence, depending on the original primary tumor.

There is evidence to suggest that given a polyclonal tumor, the interactions occurring among the clones may provide for tumor stability, as shown in B16 melanomas [33] and mouse mammary adenocarcinoma [34]. Other evidence presented shows that randomly selected primary mammary tumor transplants often produce ductal outgrowths and/or hyperplastic alveolar nodules in gland-free fat pads. However, ovarian-independent tumor transplants tend to produce in kind, suggesting greater stability of these types of tumors [10]. Therefore, if phenotypically stable tumor cells are considered to be autonomous, they might be expected to produce tumors exclusively, as did samples from tumor D (Tables 1 and 2). More labile tumors might be expected to show a more diverse growth potential, giving rise to ductal and hyperplastic outgrowths as well as tumors. Tumors B, E, F and G provided samples that exhibited this diversity in growth pattern (Tables 1 and 2).

All of the primary tumors tested were adenocarcinomas by histologic criteria. Although morphology does not give clues as to the clinical nature of adenocarcinomas, it may be relevant in predicting the maturity of the tumor according to the highly differentiated tubules and papillary structures, irregular acini or anaplastic appearance [19].

In conclusion, all data presented further support the concept of intratumor heterogeneity. It has been shown by morphology and differential growth capabilities. The same variability was observed irrespective of the type of transplant used. However, the gland-free fat pad favored ductal/HAN outgrowths, while the presence of the normal gland suppressed this type of proliferation. Tumor development was not suppressed by the intact gland and was favored in some instances. Phenotypic diversities in the expression of biological characteristics of primary tumors must take into consideration the many factors affecting the tumor cell itself, as well as tumor-host interaction [16]. The interactions among the heterogeneous constituent subpopulations tend to bring about relative phenotypic stability, thereby restricting new variant formation, as suggested by Poste and Greig [2]. These results support that concept. As the individual tumors progressed toward phenotypic autonomy and stability, they maintained their own existence with decreasing influence from the host microenvironment. This is in agreement with other studies [10, 30–32]. To bring about effective treatment of tumors, an understanding of all factors affecting tumor expression is needed.

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REFERENCES

1. Hart IR, Fidler IJ. The implications of tumor heterogeneity for studies on the biology and therapy of cancer metastasis. *Biochim Biophys Acta* 1981, **651**, 37–50.
2. Poste G, Greig R. The experimental and clinical implications of cellular heterogeneity in malignant tumors. *J Cancer Res Clin Oncol* 1983, **106**, 159–170.
3. Heppner GH, Loveless SE, Miller FR, Mahoney KH, Fulton AF. Mammary tumor heterogeneity. In: Nicolson GL, Milas L, eds. *Tumor Invasion and Metastasis*. New York, Raven Press, 1984, 209–221.
4. Heppner GH. Tumor heterogeneity. *Cancer Res* 1984, **44**, 2259–2265.
5. Fidler IJ, Hart IR. Communication: biological and experimental consequences of zonal composition of solid tumors. *Cancer Res* 1981, **41**, 3266–3267.
6. Prehn RT. Analysis of antigenic heterogeneity within individual 3-methylcholanthrene-induced mammary sarcomas. *JNCI* 1970, **45**, 1039–1045.
7. Barranco SC, Haenelt BR, Gee EL. Differential sensitivities of five rat hepatoma cell lines to anticancer drugs. *Cancer Res* 1978, **38**, 656–660.
8. Heppner GH, Dexter DL, DeNucci T, Miller FR, Calabresi D. Heterogeneity in drug sensitivity among tumor cell subpopulations of a single mouse mammary tumor. *Cancer Res* 1978, **38**, 3758–3763.
9. Dexter DL, Kowalski HM, Blazar BA, Fligiel Z, Vogel R, Heppner GH. Heterogeneity of tumor cells from a single mouse mammary tumor. *Cancer Res* 1978, **38**, 3174–3181.
10. Rivera EM, Vijayaraghavan S. Proliferation of ductal outgrowths by carcinogen-induced rat mammary tumors in gland-free mammary fat pads. *JNCI* 1982, **69**, 517–525.
11. Aidells BD, Lee AE. Transplanted cultured cells from pregnancy-dependent mammary tumors have a heterogeneous development potential. *Int J Cancer* 1979, **23**, 718–721.
12. Richards J, Nandi S. Neoplastic transformation of rat mammary cells exposed to 7,12-dimethylbenz(a)anthracene or *N*-nitrosomethylurea in cell culture. *Proc Natl Acad Sci USA* 1978, **75**, 3836–3840.
13. Miller FR, Medina D, Heppner GH. Preferential growth of mammary tumors in intact mammary fat pads. *Cancer Res* 1981, **41**, 3863–3867.
14. Vijayaraghavan S, Rivera EM. Maintenance of ovarian-dependence of carcinogen-induced rat mammary tumors serially-transplanted in parenchyma-containing mammary fat pads. *Proc AACR* 1982, **23**, 230.
15. Heppner GH, Miller BE, Miller FR. Tumor subpopulation interactions in neoplasms. *Biochim Biophys Acta* 1983, **695**, 215–226.
16. Nicolson GL. Tumor progression oncogenes and the evolution of metastatic phenotypic diversity. *Clin Expl Metastasis* 1984, **2**, 85–105.
17. DeOme KB, Miyamoto MJ, Osborn RC, Guzman RC, Lum D. Detection of inapparent nodule transformed cells in the mammary gland tissues of virgin female BALB/cfC3H mice. *Cancer Res* 1978, **38**, 2103–2111.
18. Rivera EM, Hill SD, Taylor M. Organ culture passage enhances oncogenicity of carcinogen-induced hyperplastic mammary nodules. *In Vitro* 1981, **17**, 159–166.
19. Young S, Hallowes RC. Tumors of the mammary gland. In: Turosov VS, ed. *Pathology of Tumors in Laboratory Animals*. IARC, 1973, Vol. I, 31–73.
20. Alston-Mills B, Rivera EM. Differential transplantability of enzyme-dissociated rat mammary tumor cells. *Proc AACR* 1983, **24**, 14.

21. Hager JC, Miller FR, Heppner GH. Influence of serial transplantation on the immunological-clinical correlates of BALB/cfC3H mouse mammary tumors. *Cancer Res* 1978, **38**, 2492-2500.
22. Miller BE, Miller FR, Leith J, Heppner GH. Growth interaction *in vivo* between tumor subpopulations derived from a single mouse mammary tumor. *Cancer Res* 1980, **40**, 3977-3981.
23. Miller BE, Miller FR, Heppner GH. Interactions between tumor subpopulations affecting their sensitivity to the antineoplastic agents cyclophosphamide and methotrexate. *Cancer Res* 1981, **41**, 4378-4381.
24. Schirrmacher V. Shifts in tumor cell phenotypes induced by signals from the microenvironment. *Immunobiology* 1980, **157**, 89-98.
25. Tarin D, Price JE. Influence of microenvironment and vascular anatomy on "metastatic" colonization potential of mammary tumors. *Cancer Res* 1981, **41**, 3604-3609.
26. Fidler IJ. Host microenvironment and cancer metastasis. *Proc AACR* 1983, **24**, 335.
27. Tarin D. Influence of the microenvironment on the behaviour of metastatic tumour cells: evidence from human, frog and murine tumours. *Proc AACR* 1983, **24**, 334.
28. Hager J, Fligiel S, Stanley W, Richardson AM, Heppner GH. Characterization of a variant producing cell line from a heterogeneous strain BALB/cfC3H mouse mammary tumor. *Cancer Res* 1981, **41**, 1293-1300.
29. Faulkin LJ, DeOme KB. Regulation of growth and spacing of gland elements in the mammary fat pad of the C3H mouse. *JNCI* 1960, **24**, 953-963.
30. Aidells BD, Daniel CW. Hormone-dependent mammary tumors in strain GR/A mice. II. Preneoplastic and neoplastic properties. *JNCI* 1976 **57**, 519-526.
31. Slemmer G. Interactions of separate types of cells during normal and neoplastic mammary gland growth. *J Invest Dermatol* 1974, **63**, 27-47.
32. Poste G, Doll J, Fidler IJ. Interactions between clonal subpopulations affect the stability of the metastatic phenotype in polyclonal subpopulations of B16 melamona cells. *Proc Natl Acad Sci USA* 1981, **78**, 6226-6230.
33. Nicolson GL. Cancer metastasis: organ colonization and cell surface properties of malignant cells. *Biochim Biophys Acta* 1982, **695**, 113-176.