Dynamic Aspects of Cancer Cell Populations in Metastasis

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Consideration of the entire metastatic process reveals it to be very inefficient in terms of cancer cells. Of the millions of cells released from primary cancers, relatively few metastases result. This disparity implies that in some way the process is selective. Some evidence will be reviewed that indicates that cancer cells in metastases are in some way different from those in the primary cancer from which they arose. Primary cancers and their metastases, then, should possibly be regarded as distinct entities when one is considering therapy or seeking an understanding of the fundamental aspects of metastasis. In this presentation some nonexclusive mechanisms will be discussed that could be responsible for differences between primary and secondary cancers. These include: 1) Random (statistical) selection of metastasis-forming cells; 2) The existence of genotypic metastatic subpopulations; 3) The existence of transient metastatic "compartments" within primary cancer; 4) Site-induced changes (modulation) occurring in the metastasizing cells after they arrive in the target organ; 5) A combination of the above. (Am J Pathol 97:601-608, 1979)

THE CLASSICAL PROOF that metastases arose from primary cancers was that they were histologically similar to their primary cancers but were structurally distinct from the tissues in which they grew. When other more subtle, nonmorphologic variables are examined, it is often found that metastases are in some way different from the primary tumors generating them. In view of the nature of these differences, it is interesting to speculate that had the early pathologists been biochemists, instead of histologists, the differences between the two would have been stressed rather than their similarities, and the essential relationship between primary and secondary cancers might well have been overlooked.

The evidence that cancer cells in metastases are functionally different from those in the primary lesions comes from three main types of experimental data. First, sensitivity to chemotherapeutic drugs is different in some primary cancers and some of their metastases.[1-4] Second, karyotypic analyses reveal ploidy differences between the cells of primary and secondary cancers,[5-7] which may or may not exceed the time-dependent karyotypic fluctuation in individual tumors.[8] Finally, combined *in vivo* and *in vitro* passage studies[9] indicate that cancer cells from metastases

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Presented at the Sixty-third Annual Meeting of the Federation of American Societies for Experimental Biology, Dallas, Texas, April 8, 1979.

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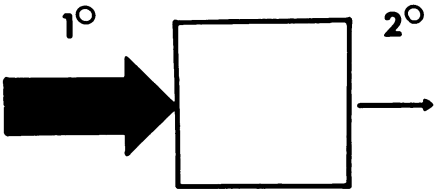
0002-9440/79/1207-0601$01.00

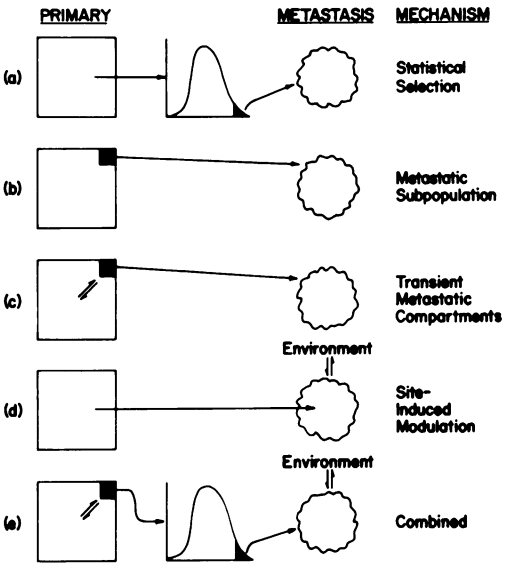
may develop or be selected out into lines that have different average biologic characteristics from the tumor from which they originally developed. A question posed by the work of Fidler,[10,11] Nicolson,[12] Tao,[13] and their colleagues is whether these various differences are exclusively explicable as manifestations of pre-existing metastatic subpopulations of cancer cells within the "wild" populations of primary cancers. In this communication I propose to examine some different possibilities to account for differences between cancer cells in a primary lesion, metastasizing cells, and cells within metastases.

It is generally accepted that the development of a metastasis is the culmination of a whole series of complex events. In order to give rise to metastases, cancer cells must not only successfully interact with their host in order to arrive at presumptive metastatic sites but also survive another sequential series of interactions that determine whether they grow at these sites.[14] The enormous disparity between the relatively large numbers of cells released from primary lesions [15,16] and the relatively small numbers of overt metastases resulting from this release indicates that metastasis is a very inefficient process (Text-figure 1). The disparity could be accounted for by the argument that the cells capable of surviving "ordeal by metastasis" comprise a small, pre-existing subpopulation within the cancer cell population as a whole (Text-figure 2b).[17-19] The isolation by cloning [10] of high and low metastatic strains of cells provides compelling evidence in favor of metastatic subpopulations. The problems as I see them are, first, not whether such subpopulations exist but whether they play a clinically significant role within the time frame of naturally occurring metastasis and, second, if they do, in which part or parts of the metastatic process such subpopulations have an advantage. As a nonexclusive alternative to subpopulations, the trauma inherent in various steps of metastasis may result in a random survival of cancer cells. Such chance survival, which is common to many biologic events, is most usefully described in statistical terms (Text-figure 2a), and the concept of subpopulations is inappropriate in this context.

Among the early events in metastasis are the release or detachment of



TEXT-FIGURE 1—The inefficiency of metastasis is indicated by the large number of cells entering the process from the primary cancer, comparatively small numbers of metastases resulting.



TEXT-FIGURE 2—Mechanisms for possible differences between cells in primary cancers and their metastases. (By permission from L Weiss: Brain Metastases. Edited by L Weiss, HA Gilbert, JB Posner. Boston, G.K. Hall, 1979)

cancer cells from the primary cancer, their dissemination in the blood and lymph vessels, their arrest by adhesion to the vascular endothelium, and the retention of a minority of them at the intravascular site long enough for them to grow or migrate out into the involved organ. Retention is a summation of cell adhesion and cell detachment. Many studies over the last twenty years or so have shown that cell adhesion and detachment are extremely sensitive to alterations in the metabolic state and in relation to proliferative and degenerative events not only in the cancer cells themselves but in surrounding tissues and the microenvironment within a "solid" tumor.[14,20,21] Thus, against a background of heterogeneity within a solid tumor, which usually consists of an uneven mixture of proliferating, dormant, dying, and dead cancer cells and other cells, *at any one time* parts of the cancer cell population are expected to be different from others, with respect to detachment, adhesion, and probably other metastasis-related functions. In this context, the concept of a *transient metastatic compartment* within a tumor (Text-figure 2c) is advanced, in which on the basis of temporary changes for which the term "subpopulation" would again be inappropriate, some cancer cells are better able to participate in and survive the rigors of the metastatic process than others. It should perhaps be emphasized that "compartment" as used here does not imply that the cells are all in one site within the tumor.

Another possibility to account for differences between primary cancers and their metastases is that they are due to interactions between organ

components and the cancer cells, after the latter have reached the site of presumptive metastasis (Text-figure 2d). One example of a site-induced change in cancer was provided by sarcoma 37 cells in mice, in which morphologic changes between the solid and ascitic forms were due to changes in cells present, rather than selective processes,[22] and were associated with reversible site-dependent changes in cell electrophoretic mobility and neuraminidase sensitivity,[23] as shown in Table 1.

More recent experiments [24] have shown that Walker 256 cancer cells growing in rats have significantly higher anodic electrophoretic mobilities when growing in the ascites form, subcutaneously or in the liver, than in the kidneys or spleen. As shown in Table 2, following incubation with neuraminidase, cells in the two latter sites lost more net surface negativity than cells growing in the other three. These differences, associated with growth in the kidney and spleen, were not explicable in terms of general differences between cancer cells growing in the ascitic and solid forms of tumors, since those growing subcutaneously or in the liver were not different from the ascites cells. The differences were also not explicable in terms of differential growth rates at the different sites. This is a matter of some potential importance, because it is known that, in some cells at least, an increased growth rate is associated with increased net surface negativity [25,26] and that the growth rate of Walker tumors varies with site.[27] In other experiments preliminary studies on two human cancers by Harlos and me have revealed that adenocarcinoma cells from hepatic metastases had net surface negativities that were 13% higher than those of cells obtained from their resected primary colonic lesions (Table 3). In this case, the secondary lesions were considerably smaller than the primary lesions and were therefore expected to be growing faster. In this case, differences between the types of lesions could thus possibly be ascribed to differential growth rate, as distinct from environmental effects per se. Analysis of the distribution of electrophoretic mobilities of the original Walker ascites tumor from which the tumors in all sites were obtained by direct injection

Table 1—Electrophoretic Mobilities of Sarcoma 37 Cells Isolated From Ascitic and Subcultaneous Tumors, With and Without Incubation With Neuraminidase[23]

	Mobilities $\mu \cdot sec^{-1} \cdot volt^{-1} \cdot cm \pm SD$		
	Ascites	Subcutaneous	Difference
Controls	-1.16 ± 0.12	-0.92 ± 0.08	$P < 0.001$
Neuraminidase	0.77 ± 0.10	0.94 ± 0.09	$P < 0.001$
P value (vertical)	< 0.001	~ 0.4	

Table 2—Mean Electrophoretic Mobilities of Walker 256 Cells From a Common Ascites Source, Grown in the Different Indicated Sites*

Site	Mobilities $\mu \cdot sec^{-1} \cdot volt^{-1} \cdot cm$ Controls	NANase-induced change
Ascites	−1.20 (100%)	−28%
Subcutaneous	−1.16 (97%)	−22%
Liver	−1.17 (98%)	−25%
Kidney	−1.12 (93%)	−8%
Spleen	−1.06 (88%)	−13%

* Each mean mobility is derived from 250 to 868 measurements, and the standard errors were all less than 0.01. The mobilities are also expressed as percentages of the ascites values. The percentage reductions in mobilities following incubation of cells from the site with neuraminidase (NANase) are also given.[24]

failed to reveal pre-existing, electrokinetic subpopulations from which the tumors at the different sites could have been derived. As long as tumors were passaged directly from kidney to kidney, or from spleen to spleen, the resulting cancer cells maintained reduced net surface negativities and reduced susceptibilities of the order shown in Table 2. However, on the first passage from kidney or spleen to the peritoneal cavity, the resulting mean electrophoretic mobilities were 99% and 98%, respectively, of cells maintained in the ascitic form, and the reductions in surface negativities following exposure to neuraminidase were 26% and 31%, respectively, compared with a 27% reduction in the cells maintained continuously in the ascites form. Thus, on electrokinetic parameters there is evidence of environmentally induced, reversible changes in these cancer cells. Such changes may well be analogous to site-induced reversible changes in cell differentiation in embryonic systems, which were termed "modulations" by P. Weiss.[28]

The precise role of surface charge in regulating cells' interactions with

Table 3—Electrophoretic Mobilities (± Standard Error) of Cancer Cells From Human Primary Adenocarcinomas of the Colon and Their Own Hepatic Metastases

Mobilities $\mu \cdot sec^{-1} \cdot volt^{-1} \cdot cm$		
Primary	Metastasis	Difference
−0.95 ± 0.02 (100)	−1.07 ± 0.02 (100)	+12.6%*
−1.05 ± 0.02 (118)	−1.19 ± 0.02 (114)	+13.0%*

* $P < 0.001$

their environment remains to be determined.[20,29] However, sialic acids, which often account for a substantial part of cell surface negativity, may influence the sensitivity of cancer cells to drugs. Thus, neuraminidase treatment of a subline of murine sarcoma 180 cells increased their resistance to 4,4′-diacetyldiphenylurea-bis(guanylhydrazone)-dimethane sulfonate (DDUG) by a factor of 2.[30] It is therefore feasible that the site-induced cell surface changes described above are associated with differences in the sensitivities of primary cancers and their metastases to chemotherapy, regardless of whether there is a causal relationship between surface ionogenic groups and drug action.

It should be emphasized that although differential sensitivity to chemotherapy has been discussed so far exclusively in terms of cancer cells, this by no means exhausts the list of possible mechanisms.[31] Thus, Donelli et al [1] studied the differential distribution of a variety of administered chemotherapeutic agents in pulmonary and lymph node metastases, found significantly higher concentrations than in their primary lesions, and concluded that these differences, which presumably reflect on blood supply, would account for differential sensitivity. Since the blood supply of tumors is itself the result of an interaction between the tumor and/or its products and adjacent host vasculature, and since the nature of the cancer cells may then be modified by growth and/or degenerative processes in response to blood supply, the importance of dynamic environmental interactions of the type shown in Text-figure 2d cannot be overstated. It is a truism that malignancy cannot be expressed in terms of cancer cells alone but can only be defined in terms of their interactions with their hosts.

It appears likely that the cancer cells in some metastases are different from those in the primary cancers from which they arose. To define the underlying causes for these largely undefined differences appears no less difficult at present than understanding original sin! Therefore, in spite of its attractiveness and novelty, I think it extremely unwise to stress the concept of a metastatic subpopulation at the expense of the other nonexclusive mechanisms outlined in Text-figure 2; and at present, I find the composite scheme outlined in Text-figure 2e the most acceptable.

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