

Metastatic Potential: Generic Predisposition of the Primary Tumor or Rare, Metastatic Variants—Or Both?

Minireview

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Metastasis is a rare event. Does it arise from rare, variant, highly metastatic cells or does a primary tumor progress to a premalignant state from which metastases arise stochastically without further changes in gene expression? Arguments and evidence have been adduced to support either position. A paper in this month's *Cancer Cell* (Kang et al., 2003) and other arguments instead suggest models combining features of both.

How do metastases arise from primary tumors? This question is of considerable importance both for clinical diagnosis and therapy and for an understanding of the underlying molecular and cellular mechanisms of cancer progression. Despite long-standing recognition of the importance of this question, we still cannot provide an adequate answer. However, our current understanding of molecular cell biology and the availability of DNA microarrays, which allow genome-wide analyses of gene expression profiles, are providing a wealth of new information relevant to the question of metastatic potential and progression. Along with the new information comes controversy about its interpretation.

Several recent analyses of human tumor material using DNA microarrays have shown that primary tumors can be classified into those with “good” or “poor” prognoses based on their patterns of gene expression (e.g., Sorlie et al., 2001). In particular, a subset of breast tumors can be identified as being predisposed to metastasis, even when no clinical evidence for metastatic spread was apparent at the time of tumor resection (van't Veer et al., 2001; van de Vijver et al., 2002). In these two papers, a “gene expression signature” was defined that predicts the probability of later appearance of metastases. It has also been reported that metastases and primary mammary tumors from the same individual show similar gene expression profiles (Perou et al., 2000) and that premalignant stages of breast cancer progression show expression profiles similar to invasive ductal carcinoma (Ma et al., 2003; Porter et al., 2003). Perhaps most surprisingly, a survey of a wide range of primary adenocarcinomas in comparison with metastases from a similar set of adenocarcinomas uncovered a “metastatic gene signature” common to many different tumor types (Ramaswamy et al., 2003). This “metastatic gene signature” could be detected in some primary tumors and had significant prognostic value.

These data all indicate that there is some global pat-

tern of gene expression in the primary tumors that is predictive of their malignant potential (see Figure 1a). That conclusion could fit with the general idea of clonal tumor progression (Nowell, 1976) toward malignancy (i.e., invasion and metastasis). Alternatively, the “good prognosis” and “poor prognosis” signatures could reflect independent evolution of the two classes of primary tumors. It has been argued further (Bernards and Weinberg, 2002; van de Vijver et al., 2002; Ramaswamy et al., 2003) that these results are in conflict with the idea that metastases arise from rare, highly metastatic variants within the primary tumor. However, by their very nature, DNA array analyses of bulk primary tumor samples cannot detect rare variant cells. Just because the array data fail to detect, and do not themselves raise the need to invoke, rare metastatic variant cells, that does not mean that such cells do not exist and play an important role. In fact, it has been shown repeatedly that cells isolated from metastases are frequently more highly metastatic than the bulk population of cells from primary tumors (Fidler and Kripke, 1977; Hart and Fidler, 1981; Fidler, 1990; Clark et al., 2000). That is, one can isolate highly metastatic variants by *in vivo* selection or merely by subcloning, and their elevated metastatic potential persists on culture. Whether these variants arise by genetic or epigenetic mechanisms, they provide evidence for an alternative model for metastatic progression (See Figure 1b).

In an elegant paper in the latest issue of *Cancer Cell*, Kang et al. (2003) present results that help to reconcile the two models. They too were investigating metastatic potential in human breast carcinoma cells. Using the human breast cancer cell line, MDA-MB-231, (originally derived from a patient with disseminated breast cancer), injected into immunodeficient mice, they were able to observe metastases to bone and to the adrenal medulla. They reisolated human cells from the osteolytic bone metastases and found that they could isolate lines of cells with stably elevated metastatic potential. Furthermore, lines selected *in vivo* for metastasis to bone or to adrenals retained their tissue selectivity. Kang et al. then performed DNA array analyses on a series of lines isolated from the bone metastases and were able to define a gene expression signature (102 genes) that correlated well with potential for osteolytic bone metastases. They also analyzed the pattern of expression of the “poor prognosis” gene signature defined by van't Veer et al. (2001) using clinical material. All the MDA-MB-231 isolates, whether of high or low metastatic potential to bone, fitted with the poor prognosis pattern. That is, the starting cell culture in the experiments of Kang et al. had already undergone the transition defined by van't Veer et al. Nonetheless, the cells could be divided into poorly and highly metastatic variants by *in vivo* selection and the highly metastatic variants had an additional “metastatic signature” overlaid on the “poor prognosis” signature.

Kang et al. went on to test whether any of the genes defined by their “bone metastasis signature” were actually causal in enhancing metastasis to bone. They over-

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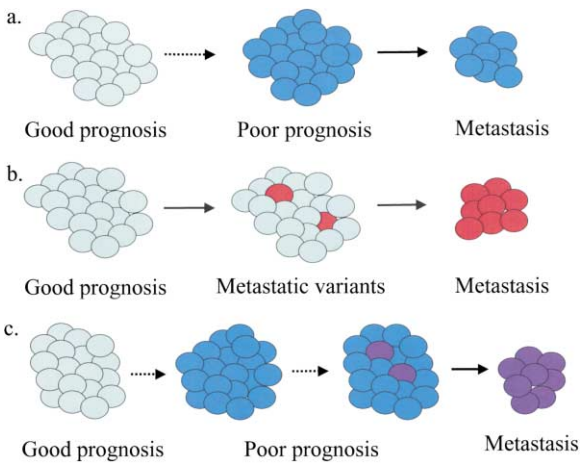


Figure 1. Contrasting Models for the Development of Metastatic Potential within Primary Tumors

(a) Several studies show that primary tumors with metastatic potential can be distinguished from those without by a difference in overall gene expression profiles, and other studies show that metastases share some patterns of gene expression with the primaries from which they originate. The simplest interpretations of these findings, diagrammed here, are (1) that there is a clonal progression from a nonmalignant state (light blue) to a malignant state (dark blue), or (2) that the two types of primary tumor arise independently with differing properties. In either case, it has been argued that metastases are rare, stochastic events not requiring further genetic change. More sophisticated versions of this model will be discussed later—see Figure 2.

(b) Other studies demonstrate the existence within primary tumors of rare, highly metastatic variants (red) that give rise to metastases. (c) Although it is sometimes argued that these two models are mutually exclusive, they are not, and a third model incorporates the two sets of data with both a global difference in the primary tumor and rare variants that eventually give metastases. The order of acquisition of the global predisposition and the appearance of variant cells is undefined. Note that the (purple) variant cells that give rise to metastases are proposed to share the global pattern of gene expression.

expressed genes singly and in combinations in the parental cells and tested for bone metastatic potential. They found a set of four genes (CXCR4, IL-11, CTGF and MMP1) from their bone-specific metastatic signature that, when coexpressed with the gene for osteopontin (which is overexpressed in both bone and adrenal metastases), enhanced metastasis to bone. Each of the five genes, when expressed alone, was insufficient to confer high metastatic potential, but, in various combinations, they elevated metastasis to levels similar to those observed with the *in vivo* selected metastatic variants. Thus, the bone metastasis signature includes genes causally involved in metastasis to bone.

An important question is whether these highly metastatic variants exist in the bulk population prior to *in vivo* selection. Kang et al. isolated subclones of the MDA-MB-231 cell line *in vitro*, without any selection, and screened them for levels of expression of the five genes they had analyzed for functional involvement. They found occasional subclones expressing 3, 4, or 5 of these genes and tested those for metastatic potential. Variants expressing 4 or 5 of the genes were highly metastatic, those expressing only 3 less so, and those

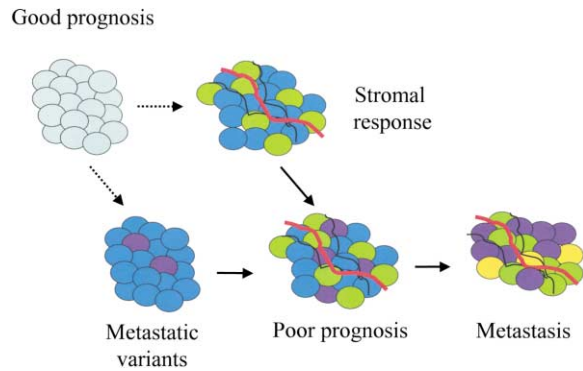


Figure 2. Multiple Steps in Metastatic Progression

Although the poor prognosis signature has generally been interpreted as reflecting a difference in the tumor cells themselves, that is unlikely to be the full picture. An alternative, though not mutually exclusive, model is that some of the differences in tumors with poor prognosis reflect infiltration of host cells (green), angiogenesis (red), or deposition of matrix (black)—collectively a “stromal response.” Any of these events could precede or follow any global change in gene expression by the tumor cells and the appearance of highly metastatic variants (purple cells) and could contribute to their survival and/or their metastatic spread. At the site of the metastasis, the tumor cells could well need and/or induce a similar stromal response to that in the primary tumor, as well as interacting with cells specific to the site of the metastasis (yellow).

For this model to be consistent with the similarity in overall gene expression profiles between metastases and tumors with poor prognosis, the metastatic variant cells (purple) should not differ greatly from the cells in the primary tumor (dark blue). In other words, the number of genes with altered expression required to generate a highly metastatic variant should be small. That has, in fact, been the observation (Clark et al., 2000; Kang et al., 2003); one to a few overexpressed genes can convert cells from poorly to highly metastatic, perhaps reflecting “priming” by the global predisposition.

expressing one or none of them were less metastatic than the bulk of the population. Therefore, the parental line (derived from a metastatic tumor) contains variant cells of high and low metastatic capacity, as well as cells with differing tissue selectivity for metastasis. These results are in complete accord with the “preexisting metastatic variant” model of Fidler. But recall that the starting cell line also showed the “poor prognosis” expression profile of van’t Veer et al. Thus, it would seem that both models are correct—in the same system (Figure 1c).

Metastasis is a complex process involving many cell biological steps and presumably requiring many changes in gene expression. Depending on the analysis conducted, one can detect subsets of these changes. The studies that looked for a gene expression signature for metastatic predisposition found one, whereas those that looked for a signature characteristic of highly metastatic cells found a different subset. It is worth noting that Kang et al. were able to distinguish cells with propensity to metastasize to bone or adrenal medulla and these two groups showed differential gene expression. In an extensive analysis of human small cell lung cancer in mice, Kakiuchi et al. (2003) recently reported differential gene expression profiles for metastases to each of four different sites (lung, liver, kidney, and bone), although they did not distinguish how much of the difference

arises from tumor responses in different environments. Thus, the metastatic signatures for metastases to different sites appear to be different.

It is well established that tumor cells and surrounding stromal cells interact and influence one another (Hanahan and Weinberg, 2000; Bissell and Radisky, 2001; Chambers et al., 2002; Fidler, 2002). Indeed, several of the genes defined by Kang et al. are likely to be involved in paracrine interactions between the metastatic mammary cells and osteoclasts in the osteolytic metastases which they studied. Because they were studying human cell lines in mice, they could distinguish which genes were expressed by the tumor cells. However, that is not true in the case of the clinical material analyzed in some of the other studies. The genes making up the “poor prognosis” (van’t Veer et al., 2001; van de Vijver et al., 2002) or “metastatic predisposition” (Ramaswamy et al., 2003) signatures could represent genes expressed by the tumor cells themselves or by “stromal” cells including vasculature, connective tissue, or immune cells. The latter model might explain the existence of a “metastatic signature” common to many tumor types. Most likely, these prognostic gene signatures reflect several distinct sorts or combinations of properties of the primary tumor, predisposing it to metastatic progression (see Figure 2). Whichever model or models apply to this predisposition difference, it seems clear that additional clonal change(s) occur to yield the final metastatic cell(s)—it is as if the global predisposition may be necessary to allow expression of the potential of the rare variants. The order of these two separable events is currently unclear and may not matter (see Figure 2). It is also possible that the relative importance of the global predisposition and the metastatic variant cells could differ among different tumor types. There is clearly still a lot we do not know about the mechanisms underlying metastasis. Neither of the simple models shown in Figures 1a and 1b explains all the data. The more complex models depicted in Figure 2 are consistent with the data but are in need of much further investigation.

The diagnostic, prognostic, and therapeutic value of the predisposition signatures of primary tumors is largely unaffected by these mechanistic considerations. However, experimental analyses such as those of Kang et al. are an essential complement, allowing investigation of additional aspects of the metastatic process that cannot readily be revealed by retrospective analyses of human tumor samples, and we can expect many new insights to come from these two complementary approaches.

Selected Reading

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