Commentary

Microarrays bring new insights into understanding of breast cancer metastasis to bone

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Abstract

Using a microarray approach, Kang and colleagues identified several genes involved in the generation of breast cancer metastasis in bone and demonstrated their roles in bone colonization *in vivo*. Their findings and interpretations are reviewed in the context of recent array studies that compared gene expression in primary tumors and metastases. RNA expression array results have already demonstrated value in predicting whether metastases will develop in patients. They have also shown that expression patterns are similar in primary tumors and metastases. The latter data have invited re-examination of long-held notions related to mechanisms of metastasis. While the arrays show promise for improving diagnostic capability in breast cancers, ascribing mechanistic interpretations to correlative data should be done with extreme caution. Kang and colleagues' paper in *Cancer Cell* elegantly reinforces the concepts that efficiency of the metastatic process is dependent on the coordinated expression of multiple genes and that the expression of metastasis-associated genes is sometimes dependent on the microenvironment in which cells find themselves.

Keywords: bone metastasis, metastatic inefficiency, metastasis genes, microarray, organotropism

Introduction

Kang and colleagues, in a recent issue of *Cancer Cell*, identified genes that promote breast carcinoma metastasis to bone [1]. Transcriptomes were compared between the parental MDA-MB-231 human breast carcinoma cell line and a variant selected one time *in vivo* for bone colonization from MDA-MB-231 (231-bone). They identified 43 overexpressed genes and 59 underexpressed genes. This pattern is referred to as a 'bone metastasis signature'. Among the overexpressed genes were matrix metalloproteinase 1, IL-11, a chemokine receptor for SDF-1 (CXCR4) and connective tissue-derived growth factor. Cotransfection of gene combinations into the parental MDA-MB-231 human breast carcinoma cell line resulted in populations as efficient at bone colonization as 231-bone, whereas transfection of individual cDNAs modestly increased the bone metastatic efficiency. Additionally, and importantly, Kang and colleagues demonstrated that bone colonizing clones pre-existed within the parental populations by single cell cloning.

Their results provide insights into the underlying mechanisms of cancer metastasis as well as support for Paget's 'seed and soil' hypothesis [2] regarding organotropism of metastases at molecular and functional levels. The findings of Kang and colleagues also contribute to the resolution of recent discussions regarding whether metastasis competent cells are rare variants or are prevalent within primary tumors. Furthermore, the findings support assertions that there are genes that specifically contribute to metastasis.

Microarrays as molecular pathology tools

Expression microarrays have the potential to revolutionize the practice of pathology by providing a molecular 'signature' that is characteristic of the cancer subtype [3,4].

231-bone = variant selected one time *in vivo* for bone colonization from MDA-MB-231; IL = interleukin.

Van't Veer and colleagues used a 70-gene set to identify and define a 'poor prognosis' transcriptome in breast cancer [5], which was subsequently used to predict the likelihood of metastasis development and patient survival [6]. One can envision a scenario whereby pharmacogenomic assays will stratify patients needing aggressive treatment (i.e. metastasis predisposed) versus less aggressive treatment (i.e. unlikely to develop microscopic metastases) [7]. Results from the *Cancer Cell* article further imply that arrays might further predict where metastases will develop. If so, therapy could be targeted to sites where metastases are possible, rather than simply administering toxins systemically.

Metastatic cells in the primary tumor: rare or predominant?

Microarray data have also been used to challenge long-held notions that metastases arise from a rare subset of cells within a primary tumor [5,6,8,9]. It is important, however, to consider the methodologies used and the interpretations that arise from the findings represented. Ramaswamy and colleagues, who compared primary tumors and metastases from multiple tumor types, found the array patterns to be nearly identical [8], leading them and other workers [5,9–11] to infer that metastatic potential is hardwired into tumor cells. As with the data of Van't Veer and colleagues, the microarrays were performed using bulk measurements (i.e. samples contained mixtures of RNA from multiple tumor cells). The samples were also 'contaminated' with normal stromal cells.

The conclusions, that a predominance of neoplastic cells had already acquired metastatic potential and that metastases arose from early oncogenic changes rather than specific events that control metastasis [9], can be challenged on the basis of other data. In short, while microarray differences are predictive of patient outcome, they neither address the issue of metastasis-competent cell prevalence [12,13] nor do they preclude the existence of metastasis-controlling genes. Likewise, microarray data cannot distinguish contributions from noncancer cells since the starting materials were not purely neoplastic cells.

Most tumors are clonal in origin, yet are heterogeneous for multiple phenotypes at diagnosis. Generation of heterogeneity is the result of genetic instability that, in turn, leads to variants with differences in metastatic potential, as demonstrated by selection of metastatic subpopulations within a tumor [12]. If one invokes the principles of Luria and Delbrück [14], the prevalence of metastatic cells within a tumor would depend on the time at which the metastatic cells emerged (i.e. earlier in progression would yield a higher proportion). While reasonable, this simple notion is complicated by the multistep nature of metastasis. Finely choreographed expression (increased or decreased) of multiple genes is required for metastatic competence. In addition, expression changes in tumor cells are superimposed by tumor cell interactions with the host microenvironment at virtually every step. If a cell is (rendered) incapable of completing any step, it is non-metastatic. In other words, every step of the metastatic cascade is rate limiting. As a result, it is not surprising that metastasis is highly inefficient [15]. Metastatic inefficiency is a critical parameter with regard to interpreting the microarray results for mechanistic insights.

Butler and Gullino, for example, showed that $(1–4) \times 10^6$ cells/g tumor per day are shed into the vasculature [16]. Shedding of cells from a primary tumor is only one step of the metastatic cascade, and the data from Butler and Gullino would argue that a substantial fraction of neoplastic cells have this ability. The ability of disseminated cells to complete subsequent steps in the metastatic cascade is not inherent *a priori*, however, as would be inferred by the relative infrequency of overt metastases. The issue of colonization is not addressed by any of the microarray data. It is known that most metastases are clonal in origin [17]; addressing the issue of metastasis genes therefore requires direct comparison of primary cells and multiple metastases. It is probable that many tumors contain subpopulations that have accumulated some, but not all, of a metastasis signature. When a tumor has a sufficient proportion of cells that express at least one of the 'poor prognosis' genes, it follows that it has increased likelihood for cells coexpressing the entire complement of metastasis-associated genes. Unfortunately, bulk array data (i.e. cells are not microdissected) cannot discern whether the appropriate pattern of expression exists within individual cells versus within the entire population.

The data from Kang and colleagues illustrate this argument elegantly. Several single cell clones showed differential expression of one or more of the bone metastasis profile genes; however, only a small fraction showed coordinate expression. Likewise, transfection of osteopontin or IL-11 alone resulted in only a modest increase in bone colonization efficiency. However, cotransfection of these genes (and others described earlier) resulted in higher metastatic efficiency. In other words, osteopontin and IL-11 were not sufficient for bone colonization. Of course, interpretation must be done with caution since there was already a baseline of 30% colonization in bone (i.e. what other genes were 'on' or 'off' already?).

This issue raises an important point that limits all experimental metastasis studies with current technology and reagents; virtually all human breast cancer cell lines were derived from metastases. Baseline measurements will be skewed as a result, but the magnitude of bias is not known. In the context of bone metastasis, it is likewise important to note that none of the commonly used human breast carcinoma cell lines were derived from bone metas-

tases (i.e. most, including MDA-MB-231, were isolated from pleural effusions). Finally, interpretation is always complicated by the artificial nature of intracardiac injection models since none of the current human breast carcinoma cell lines colonize bone from an orthotopic site. In short, xenograft models of bone metastases have serious limitations. Such issues are not unique to Kang and colleagues' article; however, they are endemic to the field. It is important to emphasize that such complications do not undermine their experiments, but point out the need for caution when interpreting the data.

Metastasis genes that control colonization

In Kang and colleagues' article, bone colonization was affected in the transfectants while adrenal colonization was not impacted by overexpression of these genes. This implies that metastasis to each organ will be characterized by different expression signatures. As a result, the concept posited earlier regarding organotropism expression signatures can be added to the hierarchy of array analysis.

The most common site of breast cancer metastasis is to the bone. This observation was the crux of Paget's seed and soil hypothesis. In short, tumor cells (the seed) must respond favorably to the tissue microenvironment (the soil) in order to form overt metastases. At the core of Paget's hypothesis is the interaction between the tumor and the host. This notion is nicely presented by Hunter and colleagues, who showed that a single oncogenic event could lead to differential metastasis, depending upon the host background [18,19].

Kang and colleagues showed that CXCR4, a chemokine receptor for the SDF-1 ligand highly expressed in bone, is more highly expressed in the 231-bone variant. Their findings are consistent with clinical data in breast cancer [20]. The prevailing hypothesis is that tumor cells respond to SDF-1 chemoattractant gradients to preferentially migrate to bone. If this hypothesis were true, then bone overexpressing SDF-1 would be more commonly colonized than bone with low expression. To the best of my knowledge, this hypothesis has not been directly tested. Nonetheless, the cumulative data emphasize that DNA expression is dependent, to some extent, upon exogenous signals. Interestingly, many of the genes expressed in 231-bone are responsive to transforming growth factor beta, which is prevalent in bone.

The conclusion that metastatic potential is determined by early oncogenic events and not metastasis-specific genes [5,6,8,9] is inconsistent with a growing literature demonstrating the existence of genes that suppress metastasis but that have no effect on tumorigenicity (reviewed in [21,22]). Studies on metastasis-promoting genes, which are the preferred targets for diagnostic studies, are complicated by the requirement for coordinated expression of multiple genes. As a result, false-negative results are more likely when determining the function of metastasis-promoting genes than the function of suppressors (since blocking any step inhibits metastasis) [23]. Studies designed to explore promoting genes therefore generally start with a baseline of metastasis and look for an increase. While entirely appropriate based upon experimental considerations, interpretation of experiments is not as straightforward as that for suppression.

Additionally, data are accumulating that some metastasis suppressors may exert organ-specific effects (i.e. growth of tumor cells is site specific). Goldberg and colleagues showed, for example, that metastasis-suppressed melanoma variants grow in the skin and disseminate to the lung. Once in the lung, however, they remain quiescent for extended periods [24]. Similarly, the Rinker-Schaeffer laboratory has shown that the metastasis suppressor MKK4 exerts a similar growth suppression at the secondary site while not affecting primary tumor growth [25,26]. In both these studies, tumor cells complete every step of the metastatic cascade except colonization. Both of these examples support findings from the Chambers and Groom laboratories showing high-frequency dissemination and extravasation, but showing low-frequency proliferation at the secondary site [27,28]. The cumulative data again emphasize the necessity for coordinated expression of genes to complete the entire metastatic process. It remains unclear at this time whether the coordination of gene expression is contemporaneous or sequential, synergistic or additive, or even whether metastatic competence is determined by unique or complementary pathways.

An unmistakable conclusion from the presented examples is that metastatic cells respond to environmental signals differently to nonmetastatic cells. It is thus easy to extrapolate that epigenetic regulation of some genes is crucial to metastasis control. The only way to address these complex issues will be to directly compare transcriptomes and proteomes in matched primary tumors and metastases (preferably from multiple sites) microdissected from adjacent normal tissues. Even then, until reliable and reproducible methods for single cell analyses are at hand, there will remain questions regarding interpretation.

In summary, the data by Kang and colleagues beautifully highlight the multigenic nature of cancer metastasis and show that some, but not all, cells in a primary tumor express the entire cadre of genes necessary to colonize bone. Their data, combined with that from many other laboratories, supports the notion that specialized subpopulations within the primary tumor can complete the metastatic process. Their data also highlight the importance of arrays for predicting clinical outcome, but emphasize the need for caution when ascribing a mechanism.

Competing interests

None declared.

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