Comparisons of Metastases in Different Organs: Biological and Clinical Implications

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Importance of Studies on Human Tumor Metastases

Tumors which acquire metastatic competence present substantial challenges to treatment and survival because this phenomenon extends the disease to other sites in the body and exponentially increases the effect of the disease on the host. Consequently, the metastatic process is the most urgent, difficult, and important issue facing cancer medicine today, both with regard to detection and to treatment. The increasing number of published investigations on the properties of metastatic neoplasms and on the underlying mechanisms indicates the higher priority now deservedly being accorded to this major problem, and the article by Wu et al. (1) in this issue of the journal is an interesting contribution to the clinical cancer literature. By comparing the expression of a selection of biomarkers in metastases in different organs from the same patient, the authors seek to determine whether the satellite tumors are essentially uniform or divergent in molecular properties. In either case, accurately drawn conclusions could have significance for understanding the underlying basis of the phenomenon. This approach, therefore, has the potential to provide novel information of mechanistic, diagnostic, and therapeutic significance and the article provides a useful opportunity for the discussion of important methodologic and interpretative considerations related to clinical and experimental research on cancer metastasis. Before sitting down with enthusiasm to consider the implications of the findings, it is worthwhile to review the difficulties of conducting penetrating research on this topic. Such initial perspective highlights the value of the approach which has been used.

Difficulties in Executing Studies on Metastasis

The relative paucity of studies on this major aspect of human cancer results from a number of conceptual and practical difficulties which must be overcome to conduct meaningful clinical and experimental studies. First, metastasis is a kinetic phenomenon occurring in a living body and cannot be modeled in vitro. It is a relentlessly parasitic phenomenon in which the malignant cell community has freed itself from normal discourse with its normal neighbors and endlessly evolves new adaptive changes which enable it to move into and dominate the terrain of other cell populations, to their detriment. Second, this evolving kinetic nature of metastasis makes it impossible to predict when it will occur and recur (metastasis from metastasis) and this complicates the collection of samples at appropriate stages, especially from human subjects. As tumor cell dissemination is a necessary but not sufficient requirement for metastasis (2–4), investigators can never be certain when it is occurring, or whether it will establish permanent new footholds, until secondary tumors are properly established. Accordingly, studies on primary and secondary tumor samples are essentially end-stage assays which can identify the pathologic and molecular characteristics of the lesions at the time of sampling but these need to be interpreted with caution with regard to previous or postulated future properties. Ideally, investigations on human clinical samples should therefore be coupled and compared with observations on the metastasis of histotypically similar human tumors in animals. The regulations governing the use of human samples are essential for the protection of human subjects, but the difficulties and sensitivities involved in obtaining informed consent for tissue procurement from patients with cancer necessarily diminish the availability of samples for research and the long time span of experiments on spontaneous metastasis in animals complicates the comparison and coordination of interpretation of results. However, progress is being made in the field and the study by Wu et al. provides a compelling illustration of how a valuable collection of clinical samples can be assembled by effective, ethically sound, organizational methods.

Evaluation of Methods and Conclusions

Although the data obtained by this work is of general interest, enthusiasm for the clinical significance of the conclusions is diminished by the techniques used for the investigation and by the interpretation of the data. The cellular and molecular heterogeneity of neoplasms has been well known for more than two decades since the initial studies by Fidler and Kripke (5, 6), which were soon substantiated in a number of human and animal tumors. Hence, the primary conclusion of the current article, embodied in its title, that metastases are heterogeneous for the expression of a few chosen molecules, is not novel, but the underlying idea of comparing several fresh tumor deposits from a series of warm autopsies could, with more work, yield a rich harvest of valuable information. It is important for the reader to recall that the behavior of cells in general, and of malignant cells in particular, is driven by the orchestration of >22,000 genes and the task for those interested in molecular mechanisms and therapy of metastasis is to sift through the noise of inconsequential gene activity to locate those features that consistently relate to the progressive malignant behavior. For this gargantuan task, a
Comparisons of Findings with Previous Work

A number of other studies on human and xenogeneic metastasis samples and matched primary tumors have been published (7–13), and some are mentioned in the text but the current authors (Wu et al.) seem completely unaware of several others or excluded them for unknown reasons. These unmentioned articles are, however, highly relevant to interpreting the work described in their report and it is necessary to briefly consider them in assessing and discussing the findings of the current work. First, the seed and soil hypothesis mentioned in the introduction to this article, which was originally advanced by Paget (14) to explain the preferential distribution of metastases from breast and other carcinomas, was unequivocally confirmed to be correct by a clinicopathologic study in ambulatory humans in 1984 (2). Other work (7–13) has shown the value of high-throughput screening techniques such as oligonucleotide microarrays (not used in this study), as a tool for the identification of candidate genes which may potentially be important for the molecular mechanisms or the therapy of cancer metastasis. These previous studies have been done with a variety of different platforms (custom glass slide arrays or commercially available Affymetrix silicon chip arrays) and the data have differed between the various reports. However, if the findings are done on commercially available platforms, which make interstudy comparisons more reliable, and are properly substantiated by subsequent fully quantitative PCR and proteomic validation, they can provide useful candidate molecules for subsequent clinical evaluation. Two such investigations (12, 13), using a screening approach with stringent independent validation of the selected molecules of interest, showed that the overall gene expression patterns are remarkably similar between primary tumors and their metastases as well as between metastases in different organs of the same host, although there are a few specific and statistically significantly different expression patterns. This illustrates how comparisons between clinically obtained human metastases and xenogeneic ones from animals can be complementary and helpful and such data need to be considered in the discussion of heterogeneity among metastases. Furthermore, a meta-analysis of all the available information to date from several laboratories (12) has indicated that the expression levels of several thousand genes are statistically very similar between metastases in different organs, but it is also evident that a few highly statistically significant differences are consistently present. The findings by all these previous teams are clearly very germane to the observations and conclusions of Wu et al. and are relevant to the evaluation of the interpretations.

Other Important Considerations in Metastasis Research Using Clinical Samples

The conclusions in the report by Wu et al. are also weakened by the absence of any information on the treatment history of the donor patients. Given that several of them survived 5 to 8 years from diagnosis, it seems unlikely that they all refused any form of treatment for such long periods. Clearly, chemotherapy and radiation therapy may alter tumor gene expression patterns and it is essential to consider this when drawing interpretations from clinical samples. It is, therefore, particularly important to guard against overinterpretation of the clinical significance of the data on such small numbers of patients and metastasis samples which were studied with rigorously systematic method of screening and testing of the properties of lesions from patients with well-characterized clinical treatment histories is needed. Given the previous body of knowledge about tumor heterogeneity, it is surprising that the authors chose such a limited number of conventional biomarkers to study and decided to use relatively subjective qualitative methods such as tissue microarrays and immunohistochemistry to obtain their data. These methods could indeed provide information on the types and location of cells expressing the chosen markers, providing suitable controls are used, but really need to be conducted in parallel with real-time reverse transcription PCR analysis and ELISA to accurately quantitate the overall expression levels when comparing metastases from different sites. The size of each of the pieces of tissue used in the tissue microarrays was stated as 1.4 mm in diameter, representing only a minute fraction of the tissue in the tumor, and there are no details on the types of controls used to ensure that there was no cross-reactivity or false positivity or negativity of staining. In contrast, the methylation analyses were more quantitative but were not analyzed statistically and the deductions based on seven apparently arbitrarily chosen genes have to be regarded as very tentative. However, the tissue samples frozen for tumor banking represent a valuable source of material for future quantitative studies using various techniques.

Clinical and Biological Significance

The findings of Wu et al. in this journal are not necessarily incompatible with those of previous investigations: it is a matter of degree. The consensus which emerges from a review of the total pool of currently available data indicates that a few tiny islands of highly significant heterogeneity of gene expression exist in an ocean of homogeneity in metastatic neoplasms, but that these may increase over time and could potentially become therapeutically significant. These global similarities as well as the rare but strikingly consistent differences are precisely documented, pictorially and numerically, in previous publications (13, 16). It is premature to judge whether these minute but significant distinctions observed between metastases in different sites represent permanent intrinsic constitutional differences between the tumor cell populations or adaptations to different environments. As experiments show (4) that tumor cells retrieved from one organ can still colonize other organs after reinoculation, at least some of the differences in expression signatures may be flexible adaptations induced by neighboring local nonneoplastic cells (16, 17). Of course, if the differential expression of even a few genes in different organ environments promotes survival and growth in those sites, then it remains possible that such small but specific deviations in expression patterns could also enable differential responses of metastases to therapy, leading to disease persistence, and therein lies the clinical significance of this type of investigation. For the potential pay-off in knowledge, further investigations on freshly excised human specimens is worth the major effort involved in obtaining and painstakingly analyzing the material.
so few probes and subjective semiquantitative methods. Indeed, some comments in their Discussion hinged on only one or two cases. Therefore, although the strategy to obtain high-quality clinical samples for analysis was well executed, the conclusions offered are speculative and not convincing.

In conclusion, it is urged that future studies on the valuable material assembled by this commendable effort by the team at Johns Hopkins should select candidate molecules for study on the basis of impartial statistical analysis of data from high-throughput screening techniques, with subsequent validation of interesting candidate molecules. They should also try to perform back-testing of conclusions regarding the importance of such molecules for mechanisms and treatment by reviewing their expression in other samples, from patients with known outcomes, in their archives. In this way, the authors could contribute to the discovery of new genes essential for metastatic cancer and organ-specific colonization as well as potential treatment targets, instead of limiting their analysis to the expression of a few known genes, some of which have been previously described as having therapeutic or diagnostic potential. The authors have a remarkable collection of samples in their possession and have the opportunity to conduct groundbreaking new studies of clinical relevance. Intensive systematic molecular analysis of this material has a high probability of yielding information of diagnostic, therapeutic, and biological value and future reports from this team are, therefore, awaited with great interest.

References
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