Germ Line Polymorphism in Metastatic Progression

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Abstract

Somatic genetic analysis of tumors and metastases has yielded a plethora of information regarding genes associated with cancer progression. However, somatic alterations in tumor cells are only one source of variability. Genetic polymorphism may also play a significant role in person-to-person variability in metastasis frequency, raising the intriguing possibility that some individuals could be predisposed to secondary tumor development. The identification and characterization of these polymorphisms may have significant implications for the development of tailored treatment or prevention of recurrent disease. (Cancer Res 2006; 66(3): 1251-4)

Metastasis and the Modifying Influence of Genetic Background

Metastasis, the source of most cancer-related mortality, is thought to result from tumor cell somatic evolution, either within the primary tumor mass or at distant sites (1). This hypothesis, while consistent with much of the available data, does not entirely explain all experimental and clinical observations. Thus, alternative hypotheses have been proposed in the literature, but none, in our opinion, entirely account for all of the observed facts (2). This suggests that although current models of metastatic progression are incomplete and too simplistic, they may all possess elements that are correct, at least in part.

One variable that may help to explain the complexity of this process is genetic background. Germ line polymorphism has long been associated with human cancer risk. Human molecular epidemiology is based on the premise that certain constitutional polymorphisms are associated with different susceptibilities. Numerous examples of this type of cancer-associated variation also exist in experimental organisms, and hundreds of modifier or quantitative trait loci have been mapped in the mouse and rat that influence a wide variety of pathologic conditions common in human populations, including disorders, such as cancer (3), diabetes (4), and hypertension (5). In addition, a number of other nonpathologic phenotypes are influenced by germ line polymorphism in model organisms (6). These results suggest that many, probably most, phenotypes have a significant genetic contribution, even traits as complex as metastasis.

To our knowledge, the best example that metastasis is not simply a somatic process came from a set of breeding experiments in our laboratory. The FVB/N-Tg(NMMTV-PyMT)854Mul transgenic mouse (PyMT) is a highly aggressive, highly metastatic mouse mammary tumor model on an inbred FVB/NJ mouse background. These animals develop mammary tumors with 100% penetrance by ~60 days of age, with ~85% to 95% developing pulmonary metastasis by 100 days of age. The modulating effects of germ line factors on metastasis were examined by introducing genetic heterogeneity into this model by breeding the PyMT transgenic animal to a variety of different inbred strains. Significant differences in metastatic frequency, with no concomitant alteration in tumor initiation or growth kinetics, were observed in heterozygous F1 progeny animals (7). This seemed to be dependent on the particular outcross, with some F1s developing ~10-fold fewer pulmonary metastases, whereas others increased pulmonary lesions by ~2- to 3-fold. Because these animals acquired the transgene by breeding and thus have the same number of copies of the PyMT antigen integrated into the same genomic site, the most likely explanation for the observed variation in metastasis frequency is the influence of germ line polymorphism.

Based on these results, a quantitative trait mapping and cloning strategy was initiated. Considering the complexity of metastasis and the number of hurdles that must be overcome for successful dissemination, it is not surprising that numerous potential loci were found to influence metastatic efficiency (8). Positional cloning strategies have recently culminated in the identification of the first of these metastasis efficiency genes. In vitro and in vivo experiments showed that a polymorphism in the Rap1 GTPase molecule Sipa1 not only significantly affected the enzymatic function of this gene, but also that relatively subtle changes in Sipa1 levels had a major effect on the metastatic potential of a highly aggressive mammary tumor cell line (9). In keeping with this, gene expression array data is consistent with SIPA1 also playing a role with human metastatic efficiency, at least in prostate cancer.

Potential Clinical Implications

The evidence that genetic modifiers of metastasis exist is therefore reasonably compelling. The question then remains, what are the implications of these genes in human cancer? Given that until recently, it has been difficult to identify those patients at increased risk for metastasis development, it is probable that the major implications of characterizing metastasis modifier genes will fall into the realm of diagnostics and prognosis. Currently, it is possible that significant numbers of patients may undergo unnecessary adjuvant treatment and are therefore experiencing needless chemotherapy-associated morbidity. However, in a
landmark article, vant’ Veer et al. (10) showed that it was possible to develop a reasonably accurate gene expression profile classifier that predicts metastatic progression using tumor-derived RNA. These findings have been shown to be reproducible (11), with a number of different laboratories reporting similar results (e.g., ref. 12).

Thus, if this technology performs well in clinical trials, oncologists will have an important new tool for tailoring individual patient treatment. There are, however, several significant shortcomings of this technology. First, it is expensive and time consuming. Second, significant lab-to-lab variability has been observed in gene expression profile experiments (13), raising concerns about the broad clinical applicability of gene expression technology. Third, it is based on tumor RNA profiling, which requires relatively large amounts of high-quality RNA from biopsy or surgical specimens to enable capture of high-quality expression data. Although advances may significantly ameliorate these difficulties, the appropriateness of using gene expression profiling in a clinical setting to predict the likelihood of secondary disease may limit the use of this potentially very important tool.

The existence of metastasis modifier genes, however, may provide an alternative to tumor gene expression profiling for prognostic testing. If it can be conclusively shown that single nucleotide polymorphisms (SNP) play an important role in metastatic efficiency in humans as well as mice, then it may be possible to develop a set of SNPs that could supplement or replace gene expression profiling as a prognostic tool. The use of SNP genotyping in this manner is advantageous for a number of reasons. For example, SNP assays have significantly less lab-to-lab and platform-to-platform variability when compared with gene expression profiling, suggesting that it might be a more reliable strategy for broad clinical use. Another crucial difference between these strategies is that because metastasis efficiency modifier genes result from germ line polymorphism, their SNP signatures exist in all tissues, which means that it would be possible to perform prognostic analysis on any tissue, including blood (Fig. 1).

To develop a SNP-based assay with adequate sensitivity and specificity, several issues will need to be addressed. In complex disorders, such as metastasis, the observed phenotype (in this instance, metastatic efficiency) is a result of multiple

Figure 1. Implication of germline polymorphism versus somatic mutation for metastasis risk assessment. A, for somatic mutation, tumor formation must have occurred to permit the isolation of tissue required for assay. B, by analyzing germ line polymorphism, risk assessment can be made using any tissue at the time of diagnosis, or even before diagnosis of primary tumor.
polymorphic genes acting in concert with environmental factors. Consequently, it is highly unlikely that any single SNP will possess 100% predictive power to determine the likelihood of metastasis development. It is more feasible that following genotyping of a panel of SNPs located within several genes, an individual would be classified as being at “higher” or “lower” risk of metastasis development. Identification of those SNPs with maximal prognostic value will prove difficult, but if this problem can be adequately addressed, the potential benefits, as outlined above, will be significant.

Further consideration of the gene expression profiling experiments described above highlights another significant implication for the use of prognostic germ line polymorphism testing. Such studies were done predicated on the hypothesis that the metastasis predictive gene expression patterns were induced by early somatic events that occurred during oncogenesis. Therefore, this strategy for identifying those patients at increased risk of metastasis would be useful only after the initiation and diagnosis of a primary tumor. Because there is evidence that dissemination may be an early, rather than a late event, this would preclude some forms of preventative action. However, because metastasis risk alleles could be screened before initiation of tumorigenesis, one can envision situations where individuals at high risk of both cancer and subsequent metastasis might be enrolled in chemoprevention regimens not only to reduce their cancer risk but also to reduce the potential burden of secondary lesions. The feasibility of this scenario was again shown in our mouse model. Chronic caffeine exposure either before or after the development of palpable mammary tumors significantly reduced metastatic burden (14), suggesting that nontoxic small agent molecules may be found that could be used for similar effects in humans.

New Perspectives on the Metastatic Process

As for our understanding of the metastatic process, the discovery of the role of constitutional polymorphism may require us to greatly broaden the concept of metastasis-associated genes. To date, such genes have been identified by comparison of primary and metastatic tumors, usually looking for differences in expression or common regions of amplification or deletion. These methods have yielded a number of interesting and important genes whose activities are significantly activated or suppressed during progression. Little consideration has, however, been given to those sets of genes in which subtle functional changes, either at the transcriptional, translational, or functional level, have a major effect on metastasis. Why have we missed them? One reason is that our model systems do not offer the opportunity to investigate the effects of genetic variation upon metastatic progression. For example, earlier studies frequently employed cell lines, many of which are derived from homozygous inbred mice, and were reimplanted into homozygous inbred mice. Thus, the opportunity to investigate the effects of genetic variation on metastatic progression has simply not been addressed. In addition, much of the focus has been on the tumor cell itself. Significant functional variation in stromal cells, mediated by allelic variation, at the site of either the primary or secondary tumor, may have major implications on whether any given disseminated cell is able to complete the metastatic cascade.

These results have also required our laboratory to expand our working definition of metastasis-related genes to encompass those genes expressed in all tissues, not just the tumor epithelium or stroma at the either primary or secondary site. Disseminating cells, by virtue of their entry into the vasculature and/or lymphatics, have the potential to interact with a staggering number of cell types, each of which may influence tumor cells in a unique manner. Because disseminated cells can reside in the individual for years or even decades, there exists the possibility that these cells are transiting through the body multiple times, acquiring new characteristics at each site until they are able to proliferate at their final location. Thus, allelic variations in genes that function in most tissues may play an important role in the success or failure of a tumor to spread.

Conclusion

Analysis of the role of germ line polymorphism in metastatic progression has the potential of enlightening us to new aspects of this critically important cancer process but also to greatly confound us by expanding the already too-complex complexity of the system. For now, the important point remains that in addition to somatic changes that contribute to the metastatic cascade, an individual's inheritance also seems to play a significant role. Exploitation of this fact may provide an important clinical tool for prognosis and prevention of the most lethal aspect of cancer.

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