Research Highlight

From Breast to the Brain: Unraveling the Puzzle of Metastasis Organotropism

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Metastatic colonization of different target organs is a highly selective process that depends on specialized properties of tumor cells. In a recent Nature paper, Massagué and colleagues built on their earlier success in functional genomic analysis of breast cancer metastasis to bone and lung and reported the identification of breast cancer brain metastasis genes, highlighting the importance of the stromal environment in the development of organ-specific metastasis.

Metastasis is a multistep process through which cancer cells disseminate from primary tumors and establish secondary lesions in distant organs. Most breast cancer-related deaths are due to the metastatic growth of tumors in vital organs such as lung, liver, brain and bone. An intriguing phenomenon in metastasis is the organotropism, i.e., the disproportional distribution of target organs for different types of cancers. For instance, over 90% of prostate cancer patients develop bone metastasis, while the colorectal cancer metastasis predominantly affects liver, but not bone or brain (Lu and Kang, 2007). The specificity of metastasis pattern can be partially attributed to the circulatory pattern. For example, liver is the first capillary bed encountered by disseminated colorectal cancer cells, which explains the high frequency of liver metastasis in colorectal cancer patients. However, several lines of evidence suggest that metastasis organotropism is influenced by factors beyond physical restraint in circulation. The common target organs of many cancer types, including breast cancer, do not have a direct circulatory connection with the primary tissues. In addition, prolonged peritoneovenous shunting treatment for ovarian cancer patients, which is intended for systematic removal of their excessive ascites but inadvertently allows the release of metastatic cancer cells in peritoneal fluid into venous circulation, usually does not cause disseminated growths of the cancer cells in the lungs (Tarin et al., 1984). Direct evidence for non-circulatory determinants of organ-specific metastasis also came from the experimental observations that different subpopulations of cancer cells originated from the same patient, when injected into mice via the same route, display distinctive organ preference for metastasis in experimental animal models (Fidler, 1973). Compatible with these observations is the century-old “seed and soil” theory which postulates that while the cancer cells (the seed) can disseminate into various organs through circulation, they can thrive only in permissive tissues (the soil) that match the intrinsic properties of the cancer cells (Paget, 1989). This theory stresses the influence of tumor-stroma interaction in shaping the development of metastasis in different organs. However, the molecular basis of “seed and soil” interactions during the development of organ-specific metastasis remains largely unknown until the post-genomic era of metastasis research.

Massagué and his colleagues in Memorial Sloan-Kettering Cancer Center have been leading the effort to harness the power of genomics to delineate the molecular basis of metastasis organotropism. Their research strategy took advantage of the natural speciation of metastasis organotropism among tumor cell variants that pre-exist in the pleural effusion of breast cancer patients. By combining genomic profiling of organotropic metastatic variants selected in vivo from animal models of breast cancer metastasis with clinical genomic studies of several large independent cohorts of breast cancer patients, the Massagué group has previously identified two multigenic signatures that mediate metastasis to bone and lung, respectively (Kang et al., 2003; Minn et al., 2005). In a recent Nature paper (Bos et al., 2009), they solved another piece of the metastasis organotropism puzzle and reported the identification and validation of genes that facilitate the development of brain metastasis. As one of the four major types of breast cancer metastasis, brain metastasis is of particular interest because of the high mortality resulted from brain lesions and their resistance to chemotherapies (Weil et al., 2005). Such resistance is explained by the blood-brain barrier (BBB), a continuous tight junction structure formed by the endothelial cells of brain parenchyma and the associated astrocytes that protects the brain from entry of foreign macromolecules and microorganisms. The BBB prevents the effective delivery of most of the chemotherapeutic drugs and antibodies into the brain, but cannot prevent the invasion of circulating metastatic cells, leading to the increasing incidence of...
brain metastasis as breast cancer patients are living longer due to the effective control of their primary tumor and metastasis in other organs. It has been unclear how cancer cells can invade into the brain and initiate metastatic growth with the BBB structure remaining intact.

In the Nature report, the authors used an experimental metastasis animal model in which two cell lines (CN34 and MDA-MB-231) established from pleural effusion of breast cancer patients, were injected into the left cardiac ventricle of immunodeficient mice to select for cell subpopulations with enhanced brain metastasis proclivity. Compared to orthotopic injection, this method directly releases the cells into the arterial circulation of the animals, and thus has the advantage of shortening the incubation time and enhancing the efficiency to produce experimental metastasis. After two rounds of in vivo selection, the resulting cell subpopulations displayed a much higher metastasis capability to the brain, but not lung or bone, than their parental cells. These cells can infiltrate the BBB and give rise to brain lesions with pathological features similar to clinical metastasis, including widespread multifocal lesions and surrounding astroglialis.

To search for brain-specific metastasis genes, comparative genomic profiling was performed and 243 genes were identified with a consistent differential expression pattern between the two matched pairs of brain-metastatic sublines and their parental counterparts. These genes were further filtered through univariate analysis by their association with brain relapse in a combined cohort of 368 human breast cancer patients. Through these analyses, a brain metastasis signature (BrMS) consisting of 17 genes was selected and was shown to be able to distinguish primary tumors with different probability of developing brain metastasis in multiple independent clinical datasets. Although BrMS expression is not restricted to certain breast cancer subtypes, it is highly enriched in ER- tumors, especially those of basal types which are known to have worse prognosis. Therefore, it would be interesting to know whether the BrMS expression pattern could serve as a prognosis factor independent of basal/luminal subtyping. Nevertheless, the robust validation of the BrMS in independent clinical samples suggests that gene signatures derived from the experimental metastasis model are also clinically relevant for the progression of human cancer. Among the 17 genes in the BrMS, COX2 and the epidermal growth factor receptor (EGFR) ligand HB-EGF were chosen for functional validation. RNAi-mediated silencing of COX2 and EGFR signaling inhibition by neutralizing antibodies significantly reduce the transmigration of cancer cells through an in vivo BBB system, and prevents brain metastasis in animals.

Comparison of the BrMS with the previously defined 18-gene lung metastasis signature (LMS) revealed a remarkable overlap between them—one-third of the genes are represented in both signatures. Consistent with this overlap is the observation that both signatures display weak but significant power to predict the metastasis event to the organ corresponding to the other signature, but are not associated with metastasis to bone, liver or lymph node. COX2 and genes encoding EGFR ligands are among the group of shared genes. It is unlikely that these genes will explain the specific brain tropism of the metastatic cells. Indeed, they were shown to mediate metastasis to lung as well (Minn et al., 2005; Gupta et al., 2007). Therefore, the authors attempted to identify genes that specifically contribute to brain metastasis. Sialyltransferase ST6GALNAC5 is expressed exclusively in the brain, but not the other normal tissues. Furthermore, ST6GALNAC5 was specifically upregulated only in clinical brain metastasis samples. ST6GALNAC5 knockdown suppressed metastasis to the brain, but not other organs. Conversely, ectopic expression of ST6GALNAC5 led to the development of small lesions in the brain from the lung-tropic LM2 cells. In vitro assay suggested that the role of ST6GALNAC5 in brain metastasis is mediated by its function to enhance the BBB permeability. However, this function alone is not sufficient for the full establishment of brain metastasis, as evidenced by the observation that ST6GALNAC5 overexpression only caused the formation of micrometastasis in the brain. Therefore, additional factors are required for further growth of tumors in the brain.

The significant overlap of BrMS with LMS, but not with the bone signature, argues for the important role of stromal environment in shaping the development of metastasis in different organs. Different to bone and liver which have a fenestrated endothelial lining to facilitate leukocyte trafficking, both lung and brain capillaries are covered with a continuous endothelial lining that is further backed by a basement membrane in lung and a tight BBB in brain. Therefore, cancer cells face the challenge to break the endothelial containment to metastasize to lung and brain. Strikingly, a large fraction of the shared genes in BrMS and LMS signatures, including COX2, ANGPTL4, LTBP1 and EGFR ligands, are known to have a function to disrupt the endothelial adhesion and increase capillary permeability. On the other hand, cancer cells face a different challenge when they metastasize to bone—they need to dissolve the calcified bone matrix, a highly specialized task that even the most malignant tumor cells are not equipped to perform. Bone tropic cancer cells generate osteolytic lesions by hijacking the normal physiological cycle of bone remodeling. A number of genes that promote the maturation of bone-degrading osteoclasts, such as IL-11, PTHrP and Osteopontin, were found to be overexpressed specifically in the bone metastatic cells (Kang et al., 2003). There is also evidence for metastasis-related environmental features only shared by bone and lung. For example, chemokine CXCL12/SDF1 is abundantly expressed in these two organs, but not in the brain (Muller et al., 2001), and accordingly, cancer cells metastatic to bone and lung specifically activate the expression of CXCR4, the receptor for CXCL12, to facilitate chemotactic homing.

Theoretically, the entire metastasis processes to various organs must share many common steps, including the early events for the cells to break away from the primary tumor and enter the circulation. Thus, metastatic cells with distinct organotropisms should manifest many common properties that are represented by the existence of “universal” metastasis genes. However, the discovery of such genes are limited by the experimental methods that directly introduce tumor cells into circulation, and genomic profiling comparisons
between subpopulations that already have a high basal level of metastatic ability. Nevertheless, several genes such as MMP1, SOX4, and members in the TGFβ pathway are commonly represented in bone, lung and brain signatures, consistent with the reported roles of these factors in the common steps of migration/invasion.

Functional analysis of organ-specific metastasis genes suggested that there might be two different categories of metastasis genes. One group of genes has an auxiliary role in tumor growth and survival in addition to their metastasis function. These genes, as exemplified by MMP1, TNSF10, PTGS2, and others, are prevalently expressed in primary tumor and have prognostic values in predicting organ-specific relapse. The other group, however, seems to only have a specific role in facilitating tumor cell invasion into the brain will be a better option to reduce the mortality from brain metastasis. Therefore, identifying the genes that bestow upon cancer cells the abilities to disseminate and breach the BBB is crucial for such clinical practice. The functional mechanism of ST6GALNAC5 in BBB infiltration still needs to be elucidated, and its potential as a therapeutic target will be an interesting topic for further research. It is also important to keep in mind that the findings presented in the series of studies by the Massagué group are likely to be just the tip of the iceberg of a potentially large number of genes that may play an important role in promoting organ-specific metastasis. Iterations of the functional genomic studies using additional cancer types and model systems are still urgently needed for a comprehensive molecular understanding of the metastasis organotropism and the translation of such knowledge into better patient care.

References