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Review

Rebuilding cancer metastasis in the mouse

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ABSTRACT

Most cancer deaths are due to the systemic dissemination of cancer cells and the formation of secondary tumors (metastasis) in distant organs. Recent years have brought impressive progress in metastasis research, yet we still lack sufficient insights into how cancer cells migrate out of primary tumors and invade into neighboring tissue, intravasate into the blood or the lymphatic circulation, survive in the blood stream, and target specific organs to initiate metastatic outgrowth. While a large number of cellular and animal models of cancer have been crucial in delineating the molecular mechanisms underlying tumor initiation and progression, experimental models that faithfully recapitulate the multiple stages of metastatic disease are still scarce. The advent of sophisticated genetic engineering in mice, in particular the ability to manipulate gene expression in specific tissue and at desired time points at will, have allowed to rebuild the metastatic process in mice. Here, we describe a selection of cellular experimental systems, tumor transplantation mouse models and genetically engineered mouse models that are used for monitoring specific processes involved in metastasis, such as cell migration and invasion, and for investigating the full metastatic process. Such models not only aid in deciphering the pathomechanisms of metastasis, but are also instrumental for the preclinical testing of anti-metastatic therapies and further refinement and generation of improved models.

1. Introduction

The most unfathomable component in tumorigenesis is the development of metastasis which accounts for more than 90% of cancer-related mortality and morbidity (Chaffer and Weinberg, 2011). Metastasis literally means "beyond stillness" and is the spread of cancer cells from the site of a primary tumor to distant anatomical sites within the body (Shibue and Weinberg, 2011). The genesis of metastasis has often been debated. In the linear progression model, one school of thought proposes that metastatic potential is a property acquired during the later stages of tumor progression by a few cancer cells due to accumulation of genetic alterations (Klein, 2009). The TNM classification of cancers (**T** describes the size of the **tumor**; **N** describes regional lymph **nodes** that are involved; **M** describes distant **metastasis**) based on the association of tumor size with increased metastasis derives from this model. In the parallel progression model, another school of thought argues that tumor cells may disseminate very early in malignant progression, colonize multiple secondary sites at different times and ultimately accumulate genetic changes independently from

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those incurred by the primary tumor (Sethi and Kang, 2011). Another interesting debate has revolved around explaining how and why cancer cells disseminate and colonize where they do. In 1889, Stephen Paget proposed the "seed and soil hypothesis" to explain the process of cancer cell dissemination (Paget, 1989). He posited that although tumor cells (seeds) are broadly disseminated during the course of malignant progression, detectable metastases only develop at those sites (soil) where the tumor cells are suitably adapted for survival and proliferation (Fidler, 2003; Fidler and Kripke, 1977). This hypothesis was challenged by Ewing in 1928 when he proposed that the anatomical layout and the circulatory patterns of the vasculature are accountable for the clinically observed occurrence of overt metastasis formation (Ewing, 1928). Both theories have stood the test of time and have been shown to contribute to the organ-specific tropism of cancer cell dissemination.

Extensive research has gone into understanding the nature and mechanisms of the 'black box' of metastasis and it has been only in the last decade that the molecular and cell-biological details of the mechanisms underlying this process have started to emerge. Also, an effective cure to curb cancer metastasis is still being explored. Hence, to be able to design effective cancer therapeutics, it is essential to model multistage carcinogenesis, including metastasis, in experimental systems. A major contributor that has helped to tease out the different facets of multistage tumorigenesis and metastasis has been the development of *in vivo* animal models that faithfully recapitulate the various stages of malignant cancer. In this review, we pay particular attention to the use and development of mouse models of metastasis that recapitulate malignant tumor progression as observed in patients and that have helped to delineate some of the molecular mechanisms underlying late stage cancer progression and metastasis.

2. Malignant tumor progression and metastasis

2.1. *Invasion-metastasis cascade*

Development and malignant progression of cancer is a multistage process. In particular, the metastases spawned by carcinomas are formed following the completion of a complex succession of cell-biological events collectively termed the invasion-metastasis cascade (Berx et al., 2007; Shibue and Weinberg, 2011). During this process, epithelial cells in the primary tumors undergo an epithelial–mesenchymal transition (EMT) and gain mesenchymal characteristics. They become migratory and invade locally through the surrounding extracellular matrix (ECM) and stromal cell layers by production of matrix metalloproteinases and other proteases. Cells eventually intravasate into the nearby blood and lymphatic vessels and then disseminate through the circulation. At a distant site, the cells may get trapped and extravasate into the tissue of a distant organ. If the secondary site is conducive, the cancer cells will generate micrometastasis and ultimately proliferate to generate macroscopic lesions (Figure 1). The multiple steps in the invasion-metastasis cascade are coordinated by molecular pathways operating within carcinoma cells as well as by heterotypic interactions between the carcinoma cells and the surrounding stromal cells, immune cells and extracellular matrix (Scheel et al., 2007).

Gene expression profiling studies with various cancer types have revealed the existence of pre-determining gene expression signatures that can predict the risk of metastatic recurrence. For instance, a set of 70 "poor prognosis gene signature" has been identified for breast cancer metastasis (van 't Veer et al., 2002; van de Vijver et al., 2002). This signature encompasses genes regulating cell cycle, invasion, metastasis and angiogenesis and is a powerful predictor of disease outcome in young patients (van de Vijver et al., 2002). In an independent study, the gene expression profiles of metastases of multiple cancer types have been compared to unmatched primary adenocarcinomas, revealing a 128 gene metastasis signature that distinguishes primary from metastatic adenocarcinomas (Ramaswamy et al., 2003).

2.2. *Metastatic organ tropism and the pre-metastatic niche*

An interesting aspect of metastasis is the proclivity of the cells of a particular cancer type to preferentially metastasize to certain organs. For instance, while breast cancer cells almost always spread to the bone, lung, brain and liver, prostrate cancer cells predominantly metastasize to the bone (Chiang and Massague, 2008). The influence of the circulatory patterns in the body and Paget's seed and soil hypothesis together may partially explain this biased organ tropism (Ewing, 1928; Paget, 1989). Yet, recent studies have led to the identification of gene signatures that define organ-specific cancer cell metastasis (Chiang and Massague, 2008; Nguyen and Massague, 2007). For instance, experiments in xenograft transplantation mouse models have identified and validated unique sets of genes that specifically promote metastasis of breast cancer cells to the bone, lung or brain (see below; Bos et al., 2009; Kang et al., 2003; Minn et al., 2005).

While the genetic and phenotypic makeup of cancer cells themselves has been conclusively shown to contribute to organ-specific metastasis, some recent elegant studies have also indicated a critical role of the tumor microenvironment in organ-specific cancer cell dissemination (Kaplan et al., 2006). For example, mouse transplantation models revealed that bone marrow-derived hematopoietic progenitor cells expressing vascular endothelial growth factor receptor-1 (VEGFR1) home to tumor type-specific "pre-metastatic sites" and form cellular clusters even before the arrival of tumor cells (Kaplan et al., 2005). In addition to specific gene signatures and the contribution of the microenvironment, metastasis of cancer cells is also influenced by activated tumor angiogenesis, by the immune system, and by epigenetic and genetic variations or polymorphisms in the cancer cell genome (Hanahan and Weinberg, 2000, 2011).

2.3. *Cellular experimental models of metastasis*

Over the years, there have been many attempts to rebuild the multiple stages of metastasis in culture systems *in vitro* and in animal models *in vivo*. These endeavors have been hampered mainly by a failure to recapitulate all the successive stages of malignant tumor progression and metastasis in one