universal and essential function of DOT1L in multiple cell types may limit its therapeutic potential unless a specific therapeutic window can be identified to distinguish normal versus leukemic cells. However, the emerging identification of the epigenetic networks and modifying enzymes deregulated by MLL fusions will no doubt continue to provide novel insights and therapeutic avenues to target these classically nondruggable oncoproteins (Figure 1).

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Inflammation Joins the "Niche"

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Bone marrow-derived cells have an important role in tumor metastasis and have been reported to "prime" distant tissues for tumor engraftment, although the mechanisms for their recruitment have remained unclear. A new study by Hiratsuka et al. describes an inflammatory signaling pathway that mediates the chemoattraction of myeloid and tumor cells to organ-specific metastatic sites.

Tumor metastasis is responsible for approximately 90% of all cancer-related deaths. It has now been well established that in order to metastasize from the primary tumor, cancer cells need to acquire additional properties that enable invasion of the extracellular matrix, intravasation, travel through blood vessels, migration to and invasion at the secondary site, and formation of metastatic nodules (Nguyen and Massague, 2007). Importantly, tumor cells are not the only participants in this complex process, as tumor-associated cells such as macrophages and bone marrow-derived progenitors have also been implicated in tumor progression and metastasis. There appears to be a collaborative role for these nonmalignant cells in enhancing metastasis, as they "precondition" the microenvironment in potential sites of metastasis, promoting tumor invasion (as reviewed in Wels et al., 2008).

The elegant early studies by Paget and Ewing first described the "seed and soil" hypothesis, suggesting that microenvironmental factors together with mechanical forces of the circulation were both important determinants of site-specific metastatic spread. Furthermore, metastatic progression is considered an evolutionary process that requires acquisition of additional genetic alterations, conferring a selective advantage to unique clones within the tumor cell population and allowing those clones to metastasize (Nguyen and Massague, 2007). Recently, studies have shown that factors derived from the primary tumor mediate the establishment of specific microenvironments in distant organs that are sites of future metastasis, the so-called "premetastatic niche." But how is it possible that alterations in the microenvironment of distant organs can occur even before the first metastatic tumor cell has arrived? The molecular pathways that underlie premetastatic niche formation are the focus of intensive ongoing study to elucidate the signaling paradigms that define future secondary tumor foci.

Over the last several years, a crucial role for bone marrow-derived cells (BMDCs) in priming distant tissues for tumor metastasis has been uncovered (Hiratsuka et al., 2006; Kaplan et al., 2005). It has been shown that cells of the hematopoietic lineage populate distant organ tissues prior to the arrival of the tumor

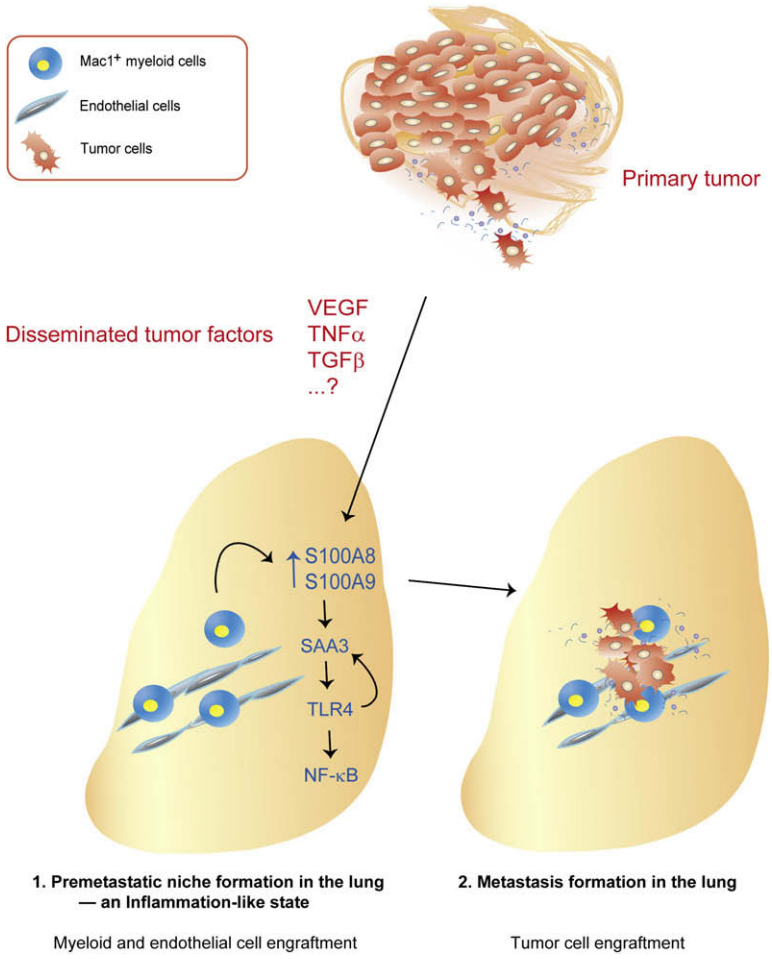

Figure 1. The Premetastatic Niche Is Similar to an Inflammatory Nidus
Both S100A8 and S100A9 induce the expression of SAA3 specifically in future metastasis sites in the lung, promoting Mac1$^+$ myeloid and endothelial cell engraftment. In this process, SAA3 promotes its own secretion by a positive feedback mechanism mediated by TLR4 and induces NF-κB signaling pathway activation (left). This inflammation-like state accelerates the future recruitment of tumor cells (right).

cells, preparing the premetastatic microenvironment (Wels et al., 2008). These BMDCs found in the premetastatic niche express several progenitor markers such as VEGFR-1 (Flt-1), c-kit, Sca-1, and CD11b, characteristic of cells of an immature status. Other molecules such as fibronectin and MMP9 are also thought to contribute to creating a highly receptive environment for circulating tumor cells. In addition to hematopoietic lineage progenitors, bone marrow-derived endothelial progenitor cells play an essential role in the formation of the metastatic niche by enabling the angiogenic switch associated with the progression of micrometastases to macrometastases. Although the molecular basis of this process is still unknown, this likely involves soluble mediators such as growth factors and chemokines secreted by cells of the primary tumor.

To date, little is known about the tumor-derived secreted factors that mediate the formation of the premetastatic niche. Hiratsuka et al. have demonstrated that primary tumor cells secrete VEGF-A, TGFβ, and TNFα, inducing the expression of the proinflammatory chemokines S100A8 (MRP8/calgranulin A) and S100A9 (MRP14/calgranulin B), in the premetastatic lung microenvironment (Hiratsuka et al., 2006). These chemoattractants increase the homing and engraftment of macrophage antigen 1 (Mac1)-expressing myeloid cells to premetastatic sites in the lung, indicating that factors released by primary tumors induce expression of chemokines within a specific metastatic target. Many details of this process, such as why these chemokines are expressed in specific foci within certain tissues, are still unclear, and further characterization is required.

In addition, Hiratsuka et al. found that engraftment of both Mac1$^+$ myeloid cells and tumor cells in lung metastatic foci appeared to be dependent on the p38-MAPK signaling pathway (Hiratsuka et al., 2006). In their current study, Hiratsuka et al. report an extension of these findings, revealing new signaling pathways and molecules involved in this process (Hiratsuka et al., 2008). In this work, serum amyloid A3 (SAA3), a protein previously implicated in phagocyte chemoattraction, is identified as a novel player. SAA3 is upregulated in specific future tumor metastatic sites by the inflammatory chemoattractants S100A8 and S100A9 (Hiratsuka et al., 2008). Functional experiments analyzing the role of these proteins indicated that SAA3 could be a downstream molecule for S100A8; however, the molecular events underlying this mechanism were not defined. Endothelial cells and Mac1$^+$ myeloid cells populating premetastatic sites within the lung appear to play critical roles in simulating an inflammation-like state that promotes migration of tumor cells to these tissues. Interestingly, SAA3 protein serves as a positive feedback regulator for chemoattractant secretion and promotes tumor cell migration (Figure 1).

The intriguing association between tumor progression and inflammation has long been a subject of research (Coussens and Werb, 2002). Nuclear factor kappa B (NF-κB) is a transcription factor that plays a central role in connecting inflammation and cancer through stimulation of proinflammatory cytokine production in myeloid and lymphoid cells (Naugler and Karin, 2008). However, the role of NF-κB signaling in premetastatic niche formation has not been previously reported.

In the present study, Hiratsuka et al. (2008) demonstrate activation of NF-κB signaling in macrophages in a SAA3-dependent fashion. This finding raises the intriguing possibility that NF-κB in the premetastatic niche could be functioning to prepare a metastatic-like environment for primary tumor cells (Figure 1). Remarkably, Toll-like receptor 4 (TLR4) seems to

be the critical receptor for SAA3 during the inflammatory-like response in the premetastatic niche. Moreover, while primary tumor cell growth was not impaired by the absence of TLR4, deletion of this receptor both dramatically decreased the number of metastatic tumor sites and reduced the engraftment of $Mac1^+$ myeloid cells in those sites. Additionally, $Mac1^+$ cell recruitment to future metastatic sites was impaired in *TLR4*$^{-/-}$ mice, suggesting that Toll-like receptor signaling may play a critical role in the crosstalk between tumor cells and BMDCs during premetastatic niche formation. Finally, inhibition of SAA3 function by neutralizing antibodies abolished cell migration to premetastatic lung sites by blocking the recruitment of both BMDCs and tumor cells, indicating that interfering with SAA3-TLR4 signaling may have a therapeutic benefit in delaying or preventing tumor metastasis.

Taken together, these data shed new light on the molecular mechanisms of premetastatic niche formation and suggest that this process is analogous to an inflammatory nidus. However, many questions still remain. For example, why does SAA3-TLR4 signaling promote the expression of inflammatory chemoattractants in some specific tissue types (such as lung) but not others? Furthermore, there may also be as yet unrecognized interactions between these cells and other inflammatory cells such as lymphocytes and fibroblasts, and also changes in the extracellular matrix. It is unclear what role other Toll-like receptors may play in the premetastatic phase, not only on immune cells but also on tumor cells, where they may influence tumor growth and host immune responses (Huang et al., 2008). It is also unclear from this study whether the proinflammatory cytokine TNFα can act independently from the SAA3-TLR4 and NF-κB pathways as shown recently by Oguma et al. (2008). These differences may emphasize the variations in the molecular events occurring during development of the primary tumor and during the establishment of metastatic disease.

Although there are many more details to be unraveled, Hiratsuka et al. have made great strides toward describing the earliest stages in metastasis formation, uncovering important roles for inflammatory signaling pathways such as p38 and NF-κB in premetastatic niche formation and in recruitment of both supportive BMDCs and disseminating cancer cells. These additional clues could be used to develop new and much-needed therapeutic strategies to prevent organ-specific metastatic spread.

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Regulating the Conversion between Rounded and Elongated Modes of Cancer Cell Movement

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Switching between elongated and rounded modes of movement allows invasive tumor cells to adapt to varying microenvironments. In a recent issue of *Cell*, Sanz-Moreno et al. identify DOCK3, NEDD9, WAVE2, and ARHGAP22 as key molecules regulating Rac and Rho signaling that determine the mode of movement driving melanoma cell metastasis.

Two properties that differentiate malignant cancer from benign are local tissue invasion by tumor cells and metastasis to sites separate from the primary tumor. In fact, metastasis is the main factor accounting for cancer treatment failure and is responsible for 90% of cancer deaths (Hanahan and Weinberg, 2000). Due to the significant impact that invasion and metastasis have on cancer mortality, intense research effort is directed at determining the critical molecular components involved, in the hope that this knowledge will eventually improve diagnosis and treatment (Olson and Sahai, 2008).

In vivo, tumor cells must break away from the primary cell mass, move through tissues by deforming and/or degrading