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

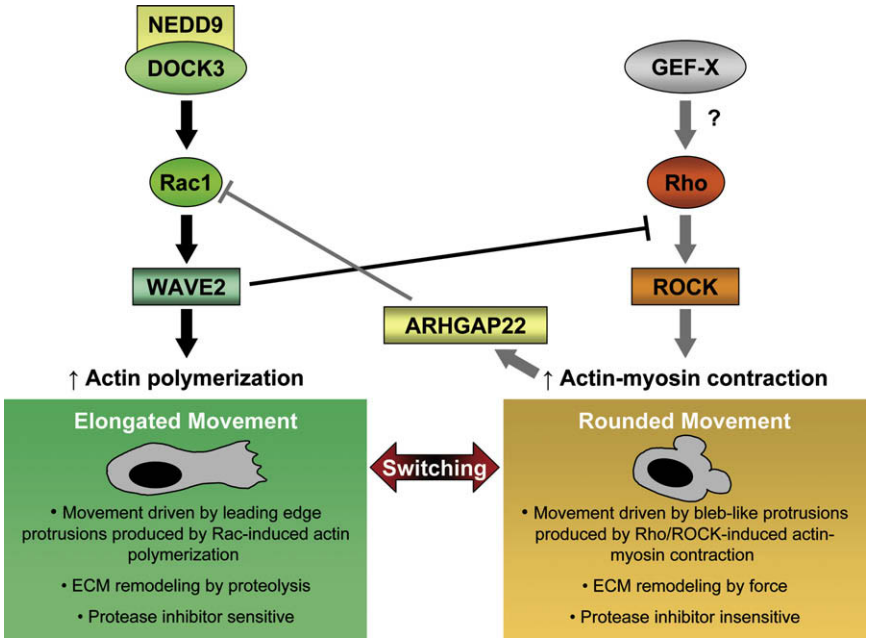

Figure 1. Switching between Elongated and Rounded Modes of Tumor Cell Movement
Reciprocal inhibitory crosstalk between Rac and Rho GTPases determines elongated or rounded modes of movement of cancer cells. Elongated ("mesenchymal") cell movement is driven by activation of Rac1 by a NEDD9/DOCK3 complex and consequent WAVE2-mediated actin polymerization. WAVE2 also represses actin-myosin contractility and inhibits rounded ("amoeboid") cell movement. In contrast, RhoA and ROCK activation lead to elevated actin-myosin contractility that promotes rounded cell movement and suppresses Rac1-dependent elongated movement by activating the ARHGAP22 RacGAP.

the extracellular matrix (ECM), cross tissue boundaries, and in the case of metastatic cells, travel via lymphatic or blood vessels to establish secondary tumors. Although modeling the complexities of the in vivo environment is a challenge, it is critically important that the models used resemble the genuine situation as closely as possible. The study of invasion and metastasis has been somewhat compromised by inadequate in vitro models; by definition, these processes occur in complex three-dimensional environments, yet cell motility has traditionally been studied using tissue culture cells grown in conditions that restrict movement to two dimensions. Not only does this overlook the importance of ECM remodeling, two-dimensional motility is the product of a single mechanistic mode in which actin polymerization at the leading edge propels the cell forward.

In a three-dimensional environment, the protrusive force of actin polymerization drives cells into an elongated, or "mesenchymal," morphology that is sufficient to push through the ECM only in the presence of protease activity to degrade the ECM (Figure 1) (Wolf et al., 2003; Sahai and Marshall, 2003). However, a second mode of motility may be observed in a three-dimensional context in which cells adopt a rounded morphology and migrate through the ECM by an "amoeboid" form of movement using actin-myosin contractile force to generate bleb-like protrusions that push and squeeze cells through the ECM without the need for proteolysis (Figure 1). Tumor cells can be classified into those that predominantly take on an elongated morphology, a rounded morphology, or a mixture of both. The tendency to adopt one specific mode or a mixture of modes is likely due to cell-intrinsic properties. Importantly, these two modes of motility have been observed in invading tumor cells in vivo (Wyckoff et al., 2006), and tumor cells may switch between the elongated and rounded modes of movement in an adaptive response to the microenvironment (Wolf et al., 2003; Sahai and Marshall, 2003). This plasticity allows cells to utilize the mode of movement most favorable to the conditions encountered as they move away from the primary tumor.

The elongated mode of movement is dependent on Rac activation, which signals to multimolecular protein complexes that promote the nucleation and elongation of actin filaments. In contrast, amoeboid movement is driven by actin-myosin contractility mediated by RhoA and its effector proteins ROCKI and ROCKII. Rac and Rho activities are mutually antagonistic: active Rac represses Rho activity and vice versa. The conversion to an active GTP-bound form is promoted by Rho guanine nucleotide exchange factors (RhoGEFs), while inactivation to the GDP-bound form is catalyzed by Rho GTPase-activating proteins (RhoGAPs) (Bos et al., 2007). Although the number of Rho GTPases is relatively modest (22), the number of RhoGEFs and RhoGAPs is far in excess of their target proteins. The multitude of Rho and Rac regulators has made it difficult to identify which are the central players in specific contexts and to determine the mechanisms of crosstalk between Rho and Rac signaling pathways.

In order to identify key proteins regulating tumor cell morphology and movement, Sanz-Moreno et al. (2008) used siRNA to screen for important RhoGEFs and RhoGAPs. A complex containing the DOCK3 RhoGEF, along with the adaptor protein NEDD9, was required for activation of Rac and consequent elongated movement. Interestingly, Rac acting via WAVE2 was found to repress phosphorylation of regulatory myosin light chain 2 (MLC2), with the consequent effect of suppressing amoeboid movement. Precisely how Rac and WAVE2, a component of a multiprotein complex that promotes actin polymerization, reduce MLC2 phosphorylation remains to be determined. In a reciprocal manner, during amoeboid movement, RhoA signaling through ROCK to promote actin-myosin contractility results in the repression of Rac activity via ARHGAP22 to suppress elongated motility. This reciprocal inhibitory crosstalk effectively increases the signal gain in favor of either specific Rho-type or Rac-type behaviors, thereby creating a bistable switch that turns on either one mode of movement or the other. This property is important because it means that as tumor cells move and encounter a series of graded variables, the response will be an either/or choice and not an uncoordinated mixture of actin cytoskeleton rearrangements. Some tumor cells may be hardwired (e.g., elevated NEDD9; Kim

et al., 2006) to stick with a particular mode of movement irrespective of the external environment, but in general, the ability to switch between modes of movement gives cells the greatest chance to react to a broad and changeable range of conditions.

Although Sanz-Moreno et al. (2008) have made a number of important discoveries, their findings also raise additional questions. Given the large number of RhoGEFs and RhoGAPs, it is notable that DOCK3/NEDD9 and ARHGAP22 are sufficient for Rac activation and inactivation, respectively. It remains to be determined whether these proteins also contribute to the movement of other types of tumor cells. RhoA is highly active in the amoeboid mode of movement, but the RhoGEF (or multiple RhoGEFs) responsible has yet to be identified. More challenging will be working out the mechanisms responsible for the inhibition of Rac activity by Rho signaling and vice versa. Although ROCK activation was sufficient to repress Rac activity, this does not appear to work via direct phosphorylation of ARHGAP22 by ROCK; instead, actin-myosin contractility regulates ARHGAP22-mediated inactivation of Rac by unknown means. Similarly, the locus of WAVE2-mediated repression of MLC2 phosphorylation has not been identified; although regulation of RhoA activity seems most likely, it is also possible that the effect occurs directly on MLC2. Given that WAVE2 is a single component of a large multiprotein complex, it will be interesting to determine whether this effect is mediated by the individual protein or the entire WAVE2 complex. It has recently been shown that depletion of the WAVE complex protein Brk1 induces blebbing, suggesting that a functional complex is required for RhoA suppression (Derivery et al., 2008).

These observations also impact upon potential antimetastatic drug therapies—if one mode of motility is targeted, for example by inhibiting protease or ROCK activity, then tumor cells may switch to the other mode. Therefore, it would be desirable to target proteins that may contribute to both modes of movement, for example LIM kinases (Scott and Olson, 2007), to increase the likelihood of successfully blocking the spread of cancer cells.

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Glycogen Synthase Kinase-3 and Cancer: Good Cop, Bad Cop?

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Dogma held that inhibition of the pleiotropic protein kinase glycogen synthase kinase-3 (GSK-3) was procarcinogenic due to its natural repression of β-catenin. Now, Wang et al. have found the reverse in certain leukemias, possibly paving the way for small-molecule GSK-3 inhibitors as selective anticancer agents.

Glycogen synthase kinase-3 (GSK-3) was originally identified and later enshrined as a member of the insulin-signaling pathway responsible for phosphorylation and inactivation of glycogen synthase, a major regulatory enzyme of glycogen metabolism. But it was soon recognized that this kinase paints with far broader strokes, influencing processes that govern cell metabolism, polarity, transcription, cell-cycle division, apoptosis, development, and cell fate. In mammals, GSK-3 exists as two isoforms, encoded by separate genes. GSK-3α (51 kDa) and GSK-3β (47 kDa) are highly conserved and widely expressed kinases that share 98% sequence identity within their catalytic domains (Doble and Woodgett, 2003). While structurally related, these isoforms are not functionally equivalent.

Unlike most protein kinases, GSK-3 is active under resting conditions and is rapidly inhibited by diverse stimuli. For example, insulin, via PI3K/Akt/PKB, induces the inactivation of GSK-3. Many of its cellular targets are held in an inactive state