Ezrin… a metastatic detERMinant?

The insidious process of tumor metastasis is the most devastating and least well-understood aspect of cancer. Metastasis is very complex and employs many cellular processes, suggesting that individual metastatic determinants may not be easily identified. Mounting evidence, culminating in the work described in two recent articles, strongly suggests that the membrane:cytoskeleton organizer Ezrin can promote tumor metastasis (Khanna et al., 2004; Yu et al., 2004). Ultimately, a better understanding of exactly how Ezrin confers metastatic advantage will provide important insight into this key problem in cancer biology.

Most cancer deaths occur due to metastatic spread. Although much is now known about how tumors initiate, our understanding of the complex process of tumor metastasis remains frustratingly vague, despite intense scientific endeavor. Normal cellular behaviors, such as survival, adhesion, and migration/invasion, are thought to be exploited by tumor cells as they metastasize (Bernards and Weinberg, 2002; Hynes, 2003 and references therein). However, few individual molecules that are known to be involved in these cellular processes have been directly implicated in tumor metastasis.

Two recent papers provide strong evidence that the membrane:cytoskeleton organizer Ezrin promotes tumor metastasis (Khanna et al., 2004; Yu et al., 2004). Together with previous work, these two studies firmly establish that high levels of Ezrin expression are linked to metastatic behavior in different types of tumors from diverse species. Furthermore, both groups provided experimental validation of a prometastatic function for Ezrin.

The ERM proteins (Ezrin, Radixin, and Moesin) and Merlin are closely related members of the band 4.1 superfamily of proteins that, when activated, interact with both membrane proteins and the actin cytoskeleton (Bretscher et al., 2002). By organizing membrane-cytoskeleton-associated complexes and creating specialized membrane domains, the ERM proteins regulate cellular activities such as survival, adhesion, and migration/invasion, all of which are important during tumor development and progression (Bretscher et al., 2002; McClatchey, 2003). Several studies suggest an important functional relationship between the ERM proteins and the small GTPase Rho, which controls actin cytoskeleton remodeling and related cellular activities. Rho-dependent phosphorylation of the ERM carboxyl-terminus constitutes the best-known mechanism of ERM activation; the ERM proteins, in turn, can regulate Rho (Bretscher et al., 2002; McClatchey, 2003).

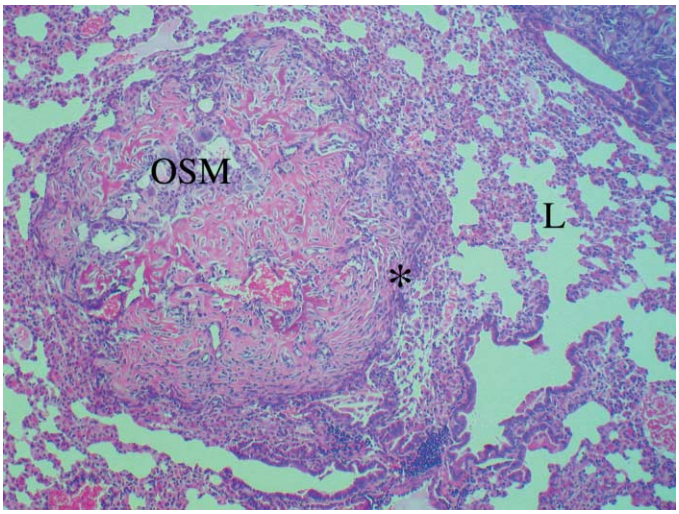

Figure 1: A well-established osteosarcoma metastasis in the lung of an *Nf2*$^{+/-}$ mouse

OSM, osteosarcoma metastasis; L, lung parenchyma. Note the boundary between the metastasis and lung parenchyma (*). Here, osteoid cells survive, proliferate, and migrate into a foreign milieu.

A powerful strategy for identifying novel metastasis-associated genes and gene expression patterns is DNA microarray analysis, which facilitates the identification of genes that contribute to metastasis through epigenetic changes in expression rather than through direct genetic mutation. Indeed, both Yu et al. and Khanna et al. began by utilizing microarray analysis to identify gene expression patterns that were specific to highly versus poorly metastatic derivatives of murine tumor cell lines (Khanna et al., 2001, 2004; Yu et al., 2004). They found that Ezrin was significantly overexpressed in highly metastatic murine rhabdomyosarcoma (RMS) and osteosarcoma (OSA) cell lines relative to their poorly metastatic counterparts. Importantly, they determined that Ezrin inhibition by stable expression of short hairpin RNA or antisense constructs directed at Ezrin, or an established dominant negative Ezrin mutant significantly reduced the metastatic capability of cell lines in both models. Conversely, overexpression of wild-type Ezrin conferred higher metastatic capability to nonmetastatic RMS cells. Finally, both groups extended their findings to other species, demonstrating clear correlations between high Ezrin levels and both increasing RMS grade in humans and poor OSA prognosis in dogs and human children. These data, together with the work of others, provide compelling evidence for a metastasis-promoting function of Ezrin (Nestl et al. 2001).

Is Ezrin a metastasis-specific gene? What is a metastasis-specific gene? It has been recently argued that specific "metastasis genes" might not exist, given that their mutation would not confer a proliferative advantage to the primary tumor. Instead, the metastatic phenotype may arise after a specific combination of genetic mutations accumulates, regardless of the sequence of their occurrence (Bernards and Weinberg, 2002). Alternatively, genetic or epigenetic changes that do not confer a proliferative advantage but specifically favor metastasis are possible. Evidence for both models exists, and both may

contribute to metastasis in the same system (Hynes, 2003). In fact, although Ezrin function has been previously linked to metastatic behavior, a direct role for any of the ERM proteins in regulating cell proliferation has not been reported to date (Bretscher et al., 2002; McClatchey, 2003 and references therein). Indeed, Ezrin levels appear to have no effect on the growth properties of the cells used in either study. In addition, Yu et al. showed that Ezrin expression progressively increases with advancing stages of human RMS. Collectively, these data argue for a prometastatic function for Ezrin specifically in late tumor progression and metastasis.

So, what advantage could Ezrin overexpression confer in the late metastatic steps of tumor progression? Using an experimental tail vein injection model of metastasis, Khanna et al. found that reducing Ezrin expression led to decreased survival of GFP-labeled highly metastatic OSA cells that reached the lung. During this rate-limiting stage of metastasis, cells must survive individually, proliferate, and utilize cell:cell and/or cell:extracellular matrix (ECM) adhesion to attach to and invade between endothelial cells (extravasate) and into the lung parenchyma—all in an environment to which they are not programmed (Figure 1). Reduced Ezrin expression was accompanied by reduced levels of active MAPK and Akt, which can both promote cell survival. Subsequent experiments suggested that activation of the MAPK but not the Akt pathway conferred a partial metastatic advantage to low Ezrin-expressing cells, suggesting that Ezrin may control multiple pathways that contribute to metastasis. Indeed, Yu et al. provided a link between the activity of the small GTPase Rho and Ezrin-dependent metastatic potential, consistent with the firmly established reciprocal relationship between the ERM proteins and Rho activity. Through its effects on the actin cytoskeleton and cell morphology, Rho is known to control cell adhesion and motility. As pointed out by both groups, Ezrin likely functions at the intersection of multiple signaling pathways.

It is interesting to note some common features shared by the two types of experimental neoplasia used in these two studies. The ERM proteins have been best studied in epithelial cells, and much less is known about their function in mesenchymal cells such as myocytes and osteoblasts, the likely precursors of RMS and OSA. In the RMS model, transgenic overexpression of hepatocyte growth factor (HGF), combined with Ink4a/Arf-deficiency, drives tumorigenesis. It is notable that nearly 100% of human osteosarcomas exhibit aberrant HGF signaling; evidence for deregulated HGF signaling in dog OSA has also been found (Birchmeier et al., 2003; MacEwen et al., 2003). Many studies have linked HGF and its receptor, c-met, to the process of tumor metastasis. Interestingly, Ezrin is a well-known effector of HGF and is required for HGF-induced migration and tubulogenesis (Bretscher et al., 2002). Indeed, Yu et al. demonstrated that Ezrin is required for branching morphogenesis by RMS cells in a similar assay. Thus, Ezrin may be specifically required for HGF/c-Met-associated tumor metastasis in this and other models.

Both RMS and OSA typically occur in pediatric and young adult patients, and are likely to arise from altered differentiation of precursor cells. Consistent with this possibility, Yu et al. also found that Six-1, a transcription factor known to control migration of muscle precursor cells during early myogenesis, is also markedly overexpressed in metastatic RMS cell lines. Interestingly, experimental overexpression of Six-1 led to increased Ezrin expression. Perhaps Ezrin functions to promote muscle precursor migration during normal development—a process that is known to be HGF-dependent (Birchmeier et al., 2003).

Finally, one must consider a possible link between Ezrin and Merlin, the product of the *NF2* tumor suppressor gene. Loss of *NF2* leads to the development of a variety of tumors in humans and mice (McClatchey, 2003). Notably, *Nf2* mutant mice are particularly predisposed to developing highly metastatic osteosarcomas (Figure 1). Overlapping subcellular localization, common interacting partners, and physical interaction between Ezrin and Merlin suggest a functional relationship. Could Ezrin overexpression inhibit Merlin function, thereby promoting tumor metastasis? Conversely, could loss of Merlin cause Ezrin activation? Future studies of Ezrin's role in tumor metastasis are likely to yield important insight into this interesting family of proteins. The identification of Ezrin as a metastatic determinant suggests that therapeutic strategies aimed at inhibiting Ezrin function may be useful in combating this complex and devastating aspect of cancer.

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