recently found to underlie a different hereditary tumor syndrome, familial leiomyomatosis and renal cell cancer (Eng et al., 2003). Isaacs et al. report in this issue that decreased activity of FH inhibits HIF hydroxylase activity (Isaacs et al., 2005). So there is an emerging picture that TCA cycle defects can impact key enzyme reactions by altering the concentration of fumarate and succinate. This is an alternative to the hypothesis that loss of function of FH or SDH subunits acts through mitochondrial dysfunction and increased generation of reactive oxygen species (Eng et al., 2003). So far, studies have focused on HIF hydroxylases, but these form only one branch of an extensive family of 2-oxoglutarate-dependent oxygenases whose activity might be modulated.

In any event, a spotlight is now on *EglN3*/*PHD3*, and it will be interesting to determine if mutations in this gene occur in pheochromocytoma, or in other tumors. An important challenge is identifying the link between the enzyme and apoptosis, which presumably involves hydroxylation of a protein other than HIF.

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Metastasis gets site specific

Organ-specific homing and colonization of cancer cells are important and interesting features of metastasis. Molecular programs that contribute to this tropism may be elucidated through gene expression profiling with DNA microarrays. Using experimentally derived breast cancer cells that home specifically to bone or to lung, several investigators have concluded that distinct alterations in gene expression underlie metastasis to these sites. Minn et al. (2005) report a set of genes involved in lung-specific metastasis of breast cancer; the authors have determined the functional contribution of several genes to the metastatic cascade, as well as the relevance of these genes to human disease.

Metastasis, the spread of cancer from the primary site of tumor growth to other organs, is the leading cause of cancer-related morbidity and mortality. It is a sequential process, contingent on tumor cell acquisition of the following capabilities: invasion, survival and arrest in the bloodstream, extravasation, and colonization at a distant site. Layered onto these general requirements of metastasis, tumor cells may acquire the capacity to preferentially colonize distinct organs. Over a century ago, Paget compared metastatic tumor cells to widely disseminated seeds, which will grow only on fertile soils (Paget, 1889). This vision propelled the hypothesis that tumor cell-host interactions serve as the prime contributor to organ-specific metastasis. An understanding of metastatic colonization, both general and organ specific, may be key to the development of antimetastatic therapies; the final step of colonization has not been completed in the majority of cancer patients at the time of diagnosis and surgery and is therefore amenable to therapeutic targeting (Steeg, 2003).

Tissue tropism or organ-specific homing of cancer cells depends on both the histology and the stage of cancer. Ocular melanomas stand as a stunning example of this tropism, metastasizing preferentially to the liver. Other cancers, such as breast, metastasize to multiple sites, most commonly lung and bone tissue and with less frequency the liver and the adrenal glands. Several factors are thought to influence the site of cancer metastasis, and these include (1) the pattern and direction of blood flow from the primary tumor, (2) mechanical trapping of tumor cells at a secondary site by small capillary beds, (3) tumor cell adhesion at a secondary site by the expression of appropriate cell surface proteins,

and finally, (4) the microenvironment of the secondary site, which can create a permissive site for metastatic colonization of tumor cells.

Based on the classical assumption that organ-specific metastasis results from alteration of distinct genetic programs and pathways, several recent studies have attempted to comprehensively define the molecular players that contribute to breast cancer tissue tropism to either the lung or the bone (Kang et al., 2003; Lee et al., 2003; Montel et al., 2005). The Massague lab established a bone metastasis model system by harvesting bone metastases of the human MDA-MB-231 breast carcinoma cell line from mice, expanding the cells in culture, and reinjecting them into mice (Kang et al., 2003). Microarray analysis of the parental versus a highly bone-metastatic line identified 102 differentially expressed genes. To test their functional contribution to bone metastasis, a limited number of these genes, encoding proteins that were largely membrane bound or secreted and thus potentially influencing tumor-host interactions, were transfected into the parental cell line. Overexpression of the chemokine receptor *CXCR4* significantly decreased bone metastasis-free survival in vivo, while Interleukin 11 (*IL-11*), which reportedly stimulated osteoclast formation among other functions, did not. The lab then took the bold step of analyzing gene combinations, reporting that *IL-11* in combination with Osteopontin (*OPN*) and connective tissue growth factor (*CTGF*) enhanced bone metastasis in vivo.

In the current issue of *Nature*, Minn et al. present elegant work that provides the next chapter in this story (Minn et al., 2005). Through a similar in vivo selection procedure, they established an MDA-MB-231-derived cell line, called LM2, that specifically colonizes the lungs. Microarray analysis revealed that the gene expression profile of the LM2 cells is distinct from the parental cells, with 95 genes exhibiting differential expression. This list was trimmed, through hierarchical cluster analysis with a spectrum of random, single-cell progeny lines, also derived from MDA-MB-231 cells that exhibit varying affinities for metastasis to the lung, to 54 genes and dubbed the "lung metastasis signature."

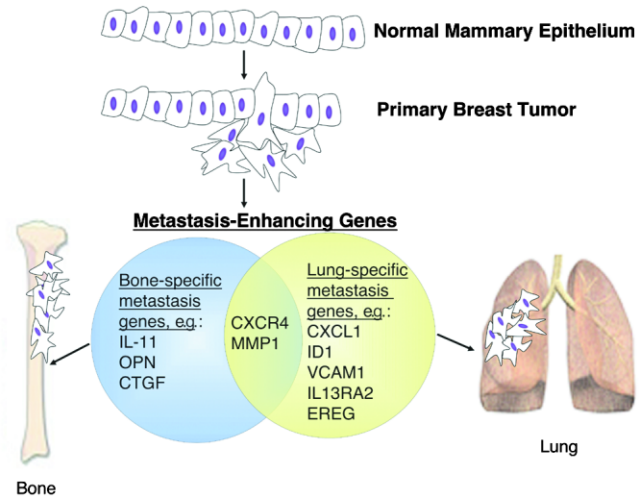

Figure 1. Schematic of breast tumor cell progression as it pertains to site-specific metastasis and colonization to bone and lung tissue

Normal mammary epithelium undergoes oncogenic transformation, and metastasis to distant organs is characterized by the expression of distinct sets of genes. The Venn diagram represents genes that contribute to homing and metastatic colonization, with genes involved in bone-specific metastasis and lung-specific metastasis and genes that are shared between the two common metastatic sites indicated.

The authors then determined the functional contribution of the genes in their signature to the process of lung metastasis. They chose nine genes from this signature, again selecting for genes that encode for membrane-localized or secreted proteins. Only overexpression of the transcription factor *ID1* by itself promoted lung metastasis of MDA-MB-231, which raises the question "is it a regulator of other genes within the signature?" In the reciprocal experiment, knockdown expression of three genes, *ID1*, the Interleukin 13 receptor *IL13RA2*, and the vascular cell adhesion molecule (*VCAM1*), significantly reduced lung colonization of the LM2 cells. Lending support to the notion that metastasis is a complex, multipathway process, transfection of combinations of genes was able to enhance lung colonization in the parental line, including the combination of the chemokine ligand *CXCL1*, Epiregulin (*EREG*), and *COX2*, none of which were significantly active as single gene transfectants. It should be noted, however, that the lung is the most common site of metastasis in most mouse assays, and that many other genes have been studied in this context on an individual basis. The degree to which these other genes mediate lung-specific metastasis is unknown.

Nor is it known to what extent the genes identified in the MDA-MB-231 model system are specific for bone or lung metastases in other model systems. The authors did demonstrate that the genes unique to the "lung metastasis signature" did not prompt bone metastasis, although metastasis to other organs was not assessed. Ten genes were found to overlap in their expression analyses of bone and lung metastasis, and these included *MMP1* and *CXCR4*. Several studies using other model systems have implicated the chemokine receptor CXCR4 in homing to both the lung and the bone (Muller et al., 2001). The data suggest that distinct sets of genes regulate homing to the lung and the bone, but some genetic components are shared (Figure 1).

The origin of metastases has been hotly debated, with at least two hypotheses posited. Whereas Fidler and colleagues contend that metastatically competent cells represent rare, preexisting variants within a primary tumor (Fidler and Kripke, 1977), several microarray studies of primary tumors have concluded that gene expression signatures present in the bulk of primary tumor cells may predict metastatic potential (Sorlie et al., 2001; van 't Veer et al., 2003). One potential reason for the predominance of metastasis-related genes in primary tumors is that they confer a selective advantage by enhancing tumorigenicity as well; thus it was hypothesized that such genes may coordinately promote growth at primary and secondary sites. Minn et al. silenced putative lung-specific metastasis genes with shRNA in the LM2 lines and injected the cells into the flanks of mice to determine if they coordinately promoted primary tumor growth (Minn et al., 2005). Only knockdown of the *ID1* gene reduced tumorigenicity; the remainder of the lung-specific genes were metastasis specific.

Minn et al. also analyzed the expression of their lung-specific metastasis signature in a cohort of 82 breast carcinomas with 10 years of clinical course follow-up (Minn et al., 2005). Expression of the lung metastasis signature by primary tumors was correlated with poor survival from lung-specific metastasis, but not

bone-specific metastasis. The importance of these data, in our opinion, lies not with the magnitude of their prognostic significance. Breast cancer is now replete with prognostic gene signatures, from young women, primary tumors versus metastases, classes of tumors, wound signatures, etc. (Chang et al., 2005; Sorlie et al., 2001; van 't Veer et al., 2003). These data confirm the relevance of the lung-specific genes identified in a single model system to the heterogeneity of human disease.

Using microarray expression analysis of primary tumors from 82 patients from Memorial Sloan-Kettering Cancer Center, the predictive value of each of the 54 genes within their experimentally derived signature was determined using a Cox proportional hazards regression model. Relatively few of the functional lung-specific genes were significant. Of the genes modulating lung-specific metastasis as single transfectants, the p values were all nonsignificant (0.569–0.833). Of the genes analyzed in combination experiments, *MMP1* and *CXCL1* retained lung-specific prognostic significance, but others, including *COX2*, *SPARC*, and *EREG*, did not. Put simply, the functional genes were not the most prognostic genes. It will be of interest to determine whether the most highly prognostic genes, such as latent TGF-β binding protein *LTBP1*, the Fascin homolog *FSCN1*, and the angiopoietin-like protein *ANGPTL4*, are functionally involved in lung-specific metastasis.

Minn et al. identified several potential players in the process of breast cancer metastasis to lung, but a detailed mechanism of organ-specific homing and colonization has yet to be established. The identification and validation of organ-specific metastatic pathways should lead to targeted therapeutics for these devastating diseases.

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Oh what a tangled web it weaves: BRCA1 and DNA decatenation

BRCA1 has significant roles in DNA repair and cell cycle checkpoint control, and is important in the maintenance of genomic stability. Defects in these pathways likely underpin the cancer susceptibility of *BRCA1* mutation carriers. Now, a new function for BRCA1 in DNA decatenation—removing the tangles introduced into chromosomes as a consequence of DNA replication—is suggested in a new paper by Lou et al. (2005) in *Nature Structural and Molecular Biology*. Ineffective DNA decatenation may lead to chromosome breakage and inappropriate repair, adding to the roll call of defects in *BRCA1* mutant cells.

A series of complex and orchestrated changes in chromosome structure are required to ensure the proper segregation of genetic material during cell division. A direct consequence of the double helical structure of DNA is that, after DNA replication during S phase, duplicated sister DNAs become topologically entangled or catenated (Wang, 2002). Sister chromatids continue to maintain a close association throughout G2 phase. Then, a signal at the onset of anaphase causes disruption of the linkage between sister chromatids, allowing them to be separated and pulled to opposite poles of the cell. However, a process called DNA decatenation needs to take place to separate chromosomes that have become entangled. This process involves DNA strand breakage and rejoining, and requires the enzyme Topoisomerase IIα (TopIIα) (Wang, 2002). Cells monitor the catenation of chromatids, and when these are insufficiently disentangled, the decatenation checkpoint is activated, arresting cells in metaphase (Deming et al., 2001). This checkpoint is separate from the response to DNA damage (Skoufias et al., 2004) and may be inactivated in some cancers (Nakagawa et al., 2004), leading to inappropriate cell cycle progression, chromosome breakage, and genomic instability.

BRCA1 is a key regulator of DNA repair and the cell cycle in higher eukaryotic cells, and dysfunction leads to predisposition to breast and a variety of other cancers (Wooster and Weber, 2003). BRCA1 is required for the efficient repair of double-strand DNA breaks (DSBs) by homologous recombination, and BRCA1 deficiency leads to the uti-