cancers. The evidence is promising, and the possibilities wide open.

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Leaving Home Early: Reexamination of the Canonical Models of Tumor Progression

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A recent report in *Science* from the Varmus laboratory (Podsypanina et al., 2008) puts an interesting twist on the origins of metastatic cells, suggesting that metastases can arise in ways that are very different from those widely believed.

The accepted canon of tumor progression involves the initial development of tumorigenicity by cells within the site of primary tumor formation. These cells remain in that site unless they become invasive, with an associated tendency to intravasate (enter blood vessels) and disseminate via the circulation to distant sites in the body. Subsequently, disseminated cells may escape blood vessels (extravasate), form micrometastases, and, with very low efficiency, succeed in forming macroscopic metastases (colonization) (Fidler, 2002, 2003; Fidler et al., 2007; Thiery, 2002).

The factors that enable the neoplastic cells within primary tumors to invade and metastasize are surely complex. Some of these determinants may already be implanted in the precursors of primary tumor cells relatively early in the course of tumor progression. For example, the differentiation program of a normal cell of origin as well as the spectrum of somatic alterations (mutations and promoter methylation events) sustained by its lineal descendants within a primary tumor are both likely to affect the probability of evolving highly malignant cell traits (Bernards and Weinberg, 2002; Ince et al., 2007). In addition, the stromal microenvironment of a primary carcinoma is also likely to contribute heterotypic signals that influence the eventual development of invasive traits (Bhowmick et al., 2004).

Once these various factors converge on individual tumor cells and impart to them an invasive phenotype, these cells may gain ready access to the systemic circulation, providing them with channels that carry them to distant sites in the body. More often than not, the destination sites are likely to be dictated by the accidental trapping of relatively large cancer cells in the small-diameter microvessels present in most organs. The lung is a favored site of initial dissemination, as its capillary bed is the first encountered by circulating tumor cells after they have entered into the venous circulation and made an initial pass through the heart.

Most cells (>95%) are cleared from sites of initial trapping in the lungs (for example) within a day or two. Moreover, the fate of the survivors that succeed in extravasating is hardly clear. Some may survive as indolent micrometastases for extended periods of time without losing their viability, while most eventually disappear. Only on very rare occasions do the cells in micrometastases succeed in proliferating vigorously and forming a macroscopic metastasis—the colonization process (Fidler, 2003).

In fact, we have only fragmentary knowledge of how cancer cells learn to colonize foreign tissue sites. Some may be endowed with this ability even prior to their leaving a primary tumor—an accidental byproduct of primary tumor progression. More frequently, however, it seems likely that cancer cells develop colonizing ability in sites of dissemination, where this phenotype is strongly selected. Once colonizing ability has been acquired, the resulting macroscopic metastases can now become sources for broadcasting a new wave of metastatic cells. The cells released in this secondary "shower" may be the most dangerous cells of all: they are doubly endowed, having the ability to both disseminate and colonize.

The acquired ability to colonize is likely to involve significant changes in cell phenotype that depend in turn on multiple changes in the genetic and epigenetic regulators of cell proliferation, motility, and invasiveness. This realization provokes another still unanswered question: Do the cells in disseminated micrometastases need to undergo many growth-and-division cycles over extended periods of time in order to generate the genetic variability that is needed in turn for the development of novel, malignancy-associated phenotypes?

Some of these issues come into play in a recent paper by Podsypanina et al. (2008). The authors developed mice with transgenes containing doxycycline-activatable *myc* and *ras* oncogenes. When fed doxycycline, these mice expressed the two oncogenes specifically in the mammary epithelium and developed diffuse mammary tumors within a period of 3 to 4 weeks. The authors turned the normal sequence of tumor progression on its head by injecting normal mouse mammary cells into the venous circulation and only activating the transgenes after these cells had lodged in the capillary beds of the lung. Hence, the cells initially arriving in the lungs were phenotypically normal and only acquired tumorigenic ability following their dissemination.

Such tail-vein injection, often termed "experimental metastasis," circumvents the initial steps of the invasion-metastasis cascade, specifically local invasion at the site of primary tumor formation and intravasation. Direct introduction into a tail vein—the normally preferred site in this protocol—causes injected cells to lodge rapidly in the lungs and thus mirrors the later steps of this cascade—survival in the circulation, extravasation, and colonization. As the authors observed, delayed activation of the transgenes after cells had lodged in the lungs yielded tumors with the histopathology and robust growth of those seen when autochthonous tumors were initiated in the orthotopic site—the mammary pad. The same outcomes were observed when mammary epithelial cells (MECs) bearing the polyoma middle T oncogene were introduced via the tail vein into the lungs and then induced to express the transgenic oncogene.

Indeed, tail-vein-injected, phenotypically normal MECs could be seeded into the lungs and allowed to persist there for a period of 16 weeks before activation of the transgenic oncogene. About 1 in 10,000 of such injected cells could become established and survive in the lungs without extensive proliferation and, with high efficiency, generate tumors following delayed, doxycycline-induced oncogene activation. Moreover, when retrieved after 4 months of residence in the lungs, the uninduced, phenotypically normal MECs could form acinar structures in three-dimensional in vitro cultures and generate mammary ductal trees when implanted in cleared mammary stromal fat pads.

These provocative observations raise many questions. Are phenotypically normal cells from one organ routinely disseminated to other sites in the human body where they may settle and persist for extended periods of time? If they do indeed survive in perfectly viable form, can they undergo the changes in the distant site needed to make them tumorigenic? And if so, do the resulting metastases resemble those formed after cells undergo transformation at a primary tumor site and acquire the ability to disperse from that site only later?

Responses to some of these questions revolve around an issue raised earlier: Does the development in a distant site of high-grade malignant traits, such as colonizing ability, require repeated rounds of cell growth and division by already disseminated cancer cells? And if so, can relatively normal cells that have been spontaneously disseminated to distant sites undergo this proliferation—i.e., in addition to surviving in organs such as the lungs, do they proliferate enough there to undergo significant genetic and epigenetic evolution?

In fact, the traditional order of invasiveness in the primary tumor as an obligatory precursor to metastatic dissemination has been upset by other experiments reported in recent years. For example, research by others on two strains of transgenic mice that are prone to develop mammary carcinomas has shown unequivocal evidence of disseminated tumor cells present in the bone marrow at a time when only atypical ductal hyperplasia (ADH, a benign stage) was present in the mammary glands of one mouse strain and occasional ductal carcinomas in situ (DCIS) and ADH in the other (Husemann et al., 2008). These other findings raise the possibility that relatively benign disseminated cancer cells can evolve into macroscopic metastases, i.e., that the genetic and epigenetic evolution of these cells can take place at sites far removed from the primary tumor. Moreover, these papers, when taken together, open our eyes to the possibility that metastatic dissemination occurs continually throughout the course of primary tumor development, yielding a diverse spectrum of disseminated cells, including ones that are at the moment of dissemination almost indistinguishable from normal cells.

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