teins, each of which have been implicated in Ras transformation of fibroblasts.

Although SPA-1 seems to be the predominant Rap1GAP in blood cell precursors, it remains possible that additional or other roles of the SPA-1 protein are involved in the in vivo effects observed by Ishida and colleagues. However, it seems likely that Rap1 is central to the effects of *SPA-1* deletion since an activated Rap1 gene, when overexpressed via retroviral transduction in primary bone marrow cells, causes a hypermyeloid phenotype. These studies need to be followed up with more phenotypic analyses, however. An additional unresolved question is the role that other Rap1GAPs might have in suppressing tumor cell growth in other tissues. As originally proposed by Altschuler and Ribeiro-Neto (1998), it seems plausible that only certain cell types are sensitive to transformation by Rap1 signaling. Also mysterious are the mechanisms that regulate Rap1's ability to suppress RasGTP activity in some contexts and deliver signals that promote growth in others. Recent data suggest that the subcellular localization of Rap1GAP activity determines whether RasGTP signaling is suppressed by Rap1, and that the two GTPases are normally activated in different subcellular regions of the cell (Ohba et al., 2003). Therefore, in some cell types and in response to the certain stimuli, Rap1 may suppress RasGTP signaling and proliferation. The rules governing the phenotypic effects of Rap1 signaling remain obscure. Some of the answers will be found when more of Rap1's downstream effectors and upstream regulators are identified, and their biological roles can be revealed in genetic experiments like those presented by Ishida and colleagues in this issue of *Cancer Cell*.

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Selected reading

Altschuler, D.L., and Ribeiro-Neto, F. (1998). Proc. Natl. Acad. Sci. USA *95*, 7475–7479.

Arai, A., Nosaka, Y., Kanda, E., Yamamoto, K., Miyasaka, N., and Miura, O. (2001). J. Biol. Chem. *276*, 10453–10462.

Cook, S.J., Rubinfeld, B., Albert, I., and McCormick, F. (1993). EMBO J. *12*, 3475–3485.

Dupuy, A.J., Morgan, K., von Lintig, F.C., Shen, H., Acar, H., Hasz, D.E., Jenkins, N.A., Copeland, N.G., Boss, G.R., and Largaespada, D.A. (2001). J. Biol. Chem. *276*, 11804–11811.

Gutmann, D.H., Saporito-Irwin, S., DeClue, J.E., Wienecke, R., and Guha, A. (1997). Oncogene *15*, 1611–1616.

Ishida, D., Kometani, K., Yang, H., Kakugawa, K., Masuda, K., Iwai, K., Suzuki, M., Itohara, S., Nakahata, T., Hiai, H., Kawamoto, H., Hattori, M., and Minato, N. (2003). Cancer Cell *4*, this issue.

Kitayama, H., Sugimoto, Y., Matsuzaki, T., Ikawa, Y., and Noda, M. (1989). Cell *56*, 77–84.

Mitelman, F., Mertens, F., and Johansson, B. (1997). Nat. Genet. *15*, 417–474.

Ohba, Y., Kurokawa, K., and Matsuda, M. (2003). EMBO J. *22*, 859–869.

Singh, L., Gao, Q., Kumar, A., Gotoh, T., Wazer, D.E., Band, H., Feig, L.A., and Band, V. (2003). J. Virol. *77*, 1614–1620.

Wienecke, R., Konig, A., and DeClue, J.E. (1995). J. Biol. Chem. *270*, 16409–16414.

York, R.D., Yao, H., Dillon, T., Ellig, C.L., Eckert, S.P., McCleskey, E.W., and Stork, P.J. (1998). Nature *392*, 622–626.

Zwartkruis, F.J., Wolthuis, R.M., Nabben, N.M., Franke, B., and Bos, J.L. (1998). EMBO J. *17*, 5905–5912.

Evidence emerges for early metastasis and parallel evolution of primary and metastatic tumors

Tumor progression to metastasis usually is assumed to occur through clonal genomic and epigenetic evolution. However, Schmidt-Kittler et al. (2003) present evidence that challenges this paradigm. They show that genomic aberrations in tumor cells disseminated in the bone marrows of patients with no clinical evidence of metastasis generally do not resemble the aberrations in the primary tumors from which they arose. They interpret this to mean that tumor cells disseminate very early and evolve to metastatic disease independent from the primary tumor. Their model suggests that adjuvant therapies should be targeted to lesions in the disseminated cells rather than lesions found in primary tumors.

A generally accepted model for tumor progression through clonal evolution is illustrated in the left portion of Figure 1. Evolutionary details are particularly well worked out in colorectal cancer (Fearon and Vogelstein, 1990). One important prediction of the clonal progression model is that the spectrum of aberrations in metastatic lesions will be similar to those in the primary tumors from which they originated since the metastases represent the end stage of evolution. Karyotypic and genomic analyses of cancers of the breast (Kuukasjarvi et al., 1997; Pandis et al., 1998), bladder (Hovey et al., 1998), colon (Al-Mulla et al., 1999), and kidney (Bissig et al., 1999) often show this feature. However, these studies also show exceptions where some metastases bear almost no genomic resemblance to the primary tumor from the same patient. Bessig et al., for example, found that ~30% of renal cell metastases were almost completely different from the primary tumors in the same patients. Likewise, Kuukasjärvi et al. (1997) found a significant fraction of breast metastases that were not strongly clonally related to the primary tumors in the same patients. They also analyzed metastases at several sites in individual patients and found substantial evolutionary divergence between these metastatic lesions and the primary tumor AND between the metastases themselves. In most cases,

metastases showed increased genome complexity compared to the primary tumors in the same patient. Taken together, these studies suggest the possibility illustrated in the right part of Figure 1, that at least for some tumors, the cells destined to form metastatic disease separate relatively early from the primary tumor and evolve independently.

Schmidt-Kittler et al. (2003) now present evidence that further challenges the clonal-progression-to-metastasis paradigm. Their study is based on comprehensive genomic analyses of primary breast tumors and single cytokeratin-positive (CK+) epithelial cells from the bone marrow of these same patients. Patients were selected that had no evidence of metastatic disease (UICC stage M0) or had metastasized (UICC stage M1). Previous publications have shown that CK+ cells originate in the tumor and their presence is associated with increased propensity to develop metastatic disease (Braun et al., 2000; Pantel et al., 1999). The CK+ cells and cells from the corresponding primary tumors were analyzed for loss of heterozygosity and genome copy number using comparative genomic hybridization as described earlier (Klein et al., 1999). The CK+ cells from M0 patients showed approximately half as many genomic aberrations as those from M1 patients. Surprisingly, most of the CK+ cells from M0 patients showed little resemblance to the primary tumors from which they presumably arose. Schmidt-Kittler et al. reasoned that this might be because the disseminated cells separated from the tumor quite early—perhaps before telomere crisis. To explore this, they compared the genomic abnormalities in disseminated cells from M0 and M1 patients. Cells from M0 patients showed whole chromosome copy number aberrations while cells from M1 patients showed subchromosomal changes characteristic of aberrations that form during telomere crisis. They attribute the eventual development of metastatic disease in M0 patients to these early disseminated cells. They suggest that these cells transition through crisis independently from cells in the primary tumor and evolve independently of the primary tumor. The long-term persistence and slow evolution of these disseminated cells would explain why metastatic disease sometimes develops years after apparently successful treatment of the primary tumor.

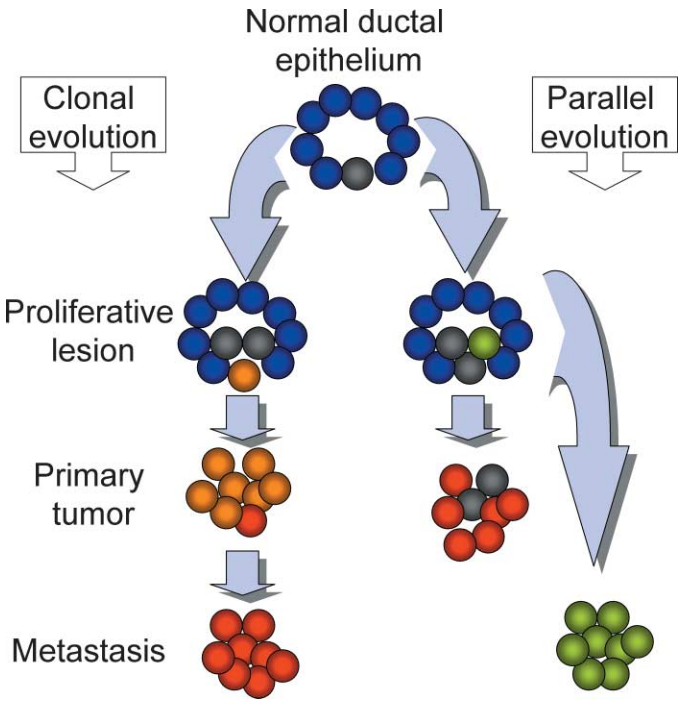

Figure 1. Schematic representation of serial and parallel models of tumor evolution to metastasis

In one model (left branch), progression to metastasis occurs through clonal evolution so that most properties of the primary tumor will be found in disseminated metastatic cells. Another model (right branch) suggests that cells that form metastatic disease separate early from the primary tumor and evolve more-or-less independently from the primary tumor.

The parallel and independent evolution model proposed by Schmidt-Kittler et al. has important clinical implications if it is correct. In particular, the model suggests that therapies that target properties of advanced primary tumors will be ineffective against metastatic cells that evolved independently after early separation from the primary tumor. Instead, selection of adjuvant therapies should be based on analyses of disseminated tumor cells rather than on the primary tumor. These cells, Schmidt-Kittler et al. argue, will show the early genetic or epigenetic events that are common to the primary tumor *and* metastases and thus are optimal therapeutic targets. While currently challenging, genomic analyses of single CK+ tumor cells are increasingly tractable and eventually might develop into a routine clinical assay. Of course, this approach to therapy selection presumes that the disseminated CK+ cells are viable and representative of those that evolve into metastatic disease. Proving this will require much additional work. If these cells do spread prior to telomere crisis as Schmidt-Kittler et al. suggest, it seems likely that most will be eliminated during crisis. In this case, genomic analyses of these cells might provide little information about the cells that do evolve into metastases. Work by Jain and colleagues (Swartz et al., 1999) also argues against the viability of disseminated tumor cells. Their murine model studies indicate that cells are shed from tumors in large numbers but have reduced clonogenicity, resistance to apoptosis, and in vivo tumorigenicity. If true in human patients, the disseminated cells might have little genomic similarity to metastases that eventually form.

In sum, Schmidt-Kittler et al. have suggested the importance of selecting therapies based on genomic analyses of disseminated tumor cells collected at the time of surgery. However, the true utility of this approach remains to be proven. Perhaps the best way to resolve this issue is by comparing genomic signatures of disseminated tumor cells taken from M0 patients with those from metastatic cells that arise years later. This will be difficult, at least in the United States, since bone marrow aspiration is not a routine part of the staging of primary breast cancer, samples of metastatic lesions are not routinely collected and, the time to acquire the appropriate matched bone marrow aspirates and metastatic tumor lesions is long. Acquisition of these resources will have to be initiated immediately in order to resolve this important issue in a timely manner.

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Selected reading

Al-Mulla, F., Keith, W.N., Pickford, I.R., Going, J.J., and Birnie, G.D. (1999). Genes Chromosomes Cancer *24*, 306–314.

Bissig, H., Richter, J., Desper, R., Meier, V., Schraml, P., Schaffer, A.A., Sauter, G., Mihatsch, M.J., and Moch, H. (1999). Am. J. Pathol. *155*, 267–274.

Braun, S., Pantel, K., Muller, P., Janni, W., Hepp, F., Kentenich, C.R., Gastroph, S., Wischnik, A., Dimpfl, T., Kindermann, G., et al. (2000). N. Engl. J. Med. *342*, 525–533.

Fearon, E.R., and Vogelstein, B. (1990). Cell *61*, 759–767.

Hovey, R.M., Chu, L., Balazs, M., DeVries, S., Moore, D., Sauter, G., Carroll, P.R., and Waldman, F.M. (1998). Cancer Res. *58*, 3555–3560.

Klein, C.A., Schmidt-Kittler, O., Schardt, J.A., Pantel, K., Speicher, M.R., and Riethmuller, G. (1999). Proc. Natl. Acad. Sci. USA *96*, 4494–4499.

Kuukasjarvi, T., Karhu, R., Tanner, M., Kahkonen, M., Schaffer, A., Nupponen, N., Pennanen, S., Kallioniemi, A., Kallioniemi, O.P., and Isola, J. (1997). Cancer Res. *57*, 1597–1604.

Pandis, N., Teixeira, M.R., Adeyinka, A., Rizou, H., Bardi, G., Mertens, F., Andersen, J.A., Bondeson, L., Sfikas, K., Qvist, H., et al. (1998). Cancer *22*, 122–129.

Pantel, K., Cote, R.J., and Fodstad, O. (1999). J. Natl. Cancer Inst. *91*, 1113–1124.

Schmidt-Kittler, O., Ragg, T., Daskalakis, A., Granzow, M., Ahr, A., Blankenstein, T.J., Kaufmann, M., Diebold, J., Arnholdt, H., Muller, P., et al. (2003). Proc. Natl. Acad. Sci. USA *100*, 7737–7742.

Swartz, M.A., Kristensen, C.A., Melder, R.J., Roberge, S., Calautti, E., Fukumura, D., and Jain, R.K. (1999). Br. J. Cancer *81*, 756–759.