A bad rap: Rap1 signaling and oncogenesis

In the paper by Ishida et al. in this issue of *Cancer Cell*, the authors report the results of targeted inactivation of a Rap1-specific GTPase-activating protein (GAP) gene, called SPA-1, in mice. Rap1 hyperactivation was observed in hematopoietic cells, which led over time to features associated with symptoms typical of human myeloid dyslastic and myeloid proliferative diseases. The authors present additional data showing that the level of Rap1 activation is important for regulating myelopoiesis and that, in the right context, can deliver an oncogenic signal.

The Rap1 gene was discovered in a tissue culture-based genetic screen as a suppressor of Ras transformation in rodent fibroblasts (Kitayama et al., 1989). Indeed Rap1, when expressed at high levels, can compete with Ras signaling by binding to but not activating Ras effector molecules, such as c-Raf (Cook et al., 1993). However, more physiologically relevant activation of endogenous Rap1, by growth factors, does not seem to repress Ras-mediated activation of ERK (Zwartkruis et al., 1998). Moreover, expression of Rap1 at high levels can morphologically transform Swiss 3T3 fibroblasts, causing increased saturation density and decreased doubling time (Altschuler and Ribeiro-Neto, 1998). Finally, in certain cell lines, Rap1 is capable of activating ERK via activation of B-raf (York et al., 1998). Since these early discoveries, many scientists, after years of work, have not yet determined whether Rap1 normally antagonizes Ras signaling or can be a "bad actor" in cancer, delivering an oncogenic signal, just as Ras does. Indeed, Rap1 may do both things. The paper by Ishida et al. in this issue of *Cancer Cell* provides evidence suggesting that Rap1 can deliver an oncogenic signal in vivo, may do so in collaboration with RAS/ERK signaling, and is especially important for maintaining normal myelopoiesis.

The authors present data on the effects of targeted disruption of a Rap1-specific GTPase-activating protein (GAP) gene called *SPA-1* in mice. At about one year of age, *SPA-1*$^{-/-}$ mice develop one of three related disorders resembling human chronic myeloproliferative or myelodysplastic diseases. Some of the mice developed myelodysplastic disease with pancytopenia and abnormal granulocytes and megakaryocytes present in circulation. Another group had peripheral leukocytosis with circulation of immature blast-like cells of various lineages. In at least some of these mice, mono or oligoclonality of B lineage blast cells could be documented. A third group developed a chronic myeloproliferative disease. *SPA-1*$^{-/-}$ preleukemic lineage-negative bone marrow cells showed increased Rap1 and ERK activation without any change in Ras activation. However, the blast crisis disease that develops in *SPA-1*$^{-/-}$ mice was associated with increased basal Rap1 *and* RasGTP levels. These data suggest that Ras activation might cooperate with Rap1 activation in disease progression in this setting. Nevertheless, Rap1 activation seems to be critical for maintenance of the transformed state, since transduction of the *SPA-1* gene into a *SPA-1*$^{-/-}$ myeloid leukemia cell line inhibited Rap1 activation and leukemogenicity in SCID mice without affecting RasGTP or activated ERK levels. Furthermore, transduction of normal marrow cells with an activated form of Rap1 resulted in enhanced proliferation in vivo. The spectrum of hematologic disease seen in *SPA-1*$^{-/-}$ mice is remarkable and suggests that Rap1GTP plays a vital role in controlling normal hematopoiesis. These data also help to explain the finding that a Rap1-specific guanine nucleotide exchange factor, called RasGRP2 or CAL-DAG-GEF I, is activated by proviral insertion in a subset of BXH-2 strain acute myeloid leukemias (Dupuy et al., 2001). Taken together, these data suggest that Rap1 activation may be a general feature of myelodysplasia, myeloid leukemia, and possibly other human cancers.

Does Rap1 signaling play a role in human myeloid leukemia and contribute to cancer in other settings? Certainly the data provided by Ishida and colleagues (2003) demonstrate that *SPA-1* might be a myeloid tumor suppressor gene, and so changes in *SPA-1* gene expression in primary leukemic cells should be sought. The *SPA-1* gene maps to human chromosome 11q13, a site frequently altered in human leukemia (Mitelman et al., 1997). Other suggestive evidence for a role of Rap1 in cancer comes from study of oncogenic human papillomaviruses (HPV). A cellular Rap1GAP, called E6TP1, is targeted for ubiquitin-mediated degradation by the viral E6 oncoprotein, suggesting that resultant hyperactivation of Rap1 might contribute to cervical and other cancers associated with chronic HPV infection (Singh et al., 2003). Another bit of suggestive evidence comes from the study of a human tumor predisposition syndrome called tuberous sclerosis type 2 (TSC-2). Inherited as an autosomal, dominant disease, somatic inactivation of *TSC2* predisposes to benign and rarely malignant tumors. Tuberin, the product of the *TSC2* gene, encodes a Rap1GAP (Wienecke et al., 1995). Interestingly, sporadic glioma often shows loss of tuberin expression or increased Rap1 expression (Gutmann et al., 1997).

How does Rap1 signaling contribute to cancer? Two likely scenarios are activation of a B-Raf/MEK/ERK signaling pathway or integrin activation. Cell biological and genetic studies suggest that Rap1 is involved in delivering a signal leading to integrin activation and increased cell adhesion (Arai et al., 2001). This could in turn lead to increased "outside-in" signaling mediated by engagement of integrins by their ligands. The net effect may be that hematopoietic cells more easily survive outside of their normally occupied niches in the bone marrow. In time, this could lead to the effects observed in the *SPA-1*$^{-/-}$ mice. Presumably, additional environmental or genetic events provide signals required for more aggressive disease. In particular, Ishida et al. observed high levels of Ras activation in the blast crisis leukemia that developed in some mice. These data suggest that leukemogenesis may be associated with the coordinate dysregulation of multiple Ras superfamily members, which provides the correct balance of phenotypic effects to maintain the leukemic cell phenotype. Either Rap1 or Ras activation may occur during the preleukemic period, requiring different subsequent events for disease progression. Other Ras superfamily members that seem to play a role in cancer include Rac, Cdc42, and Rho pro-

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,