Cell-autonomous and -nonautonomous contributions of STAT1 in murine models of tumorigenesis

In this issue of *Cancer Cell*, Kovacic and colleagues have reexamined the role of STAT1 in murine models of leukemogenesis. Their studies shed new light on the complex interplay between cell-autonomous contributions and host responsiveness to cancer and elucidate a previously unknown role of STAT1 in tumor progression.

Available evidence has indicated that STAT1 has tumor suppressor activity, in that more rapid tumor progression has been observed in *STAT1*-deficient mice (e.g., Lesinski et al., 2003; Shankaran et al., 2001). In consonance with previous reports, Kovacic et al. (2006) show that *v-abl*-mediated transformation of hematopoietic cells derived from *STAT1*$^{-/-}$ animals contributes to increased number of growth factor-independent pro-B colonies in vitro.

However, when low numbers of cells from *v-abl*-transformed pro-B cell lines were injected into immunodeficient *RAG2*$^{-/-}$ recipient mice, *STAT1* deficiency impaired rather than enhanced leukemia progression in vivo. These findings suggested cell-nonautonomous contributions to disease development, with innate immune surveillance as a potential mechanism. Kovacic and colleagues demonstrated, consistent with the published literature (Lee et al., 1999), that *STAT1*$^{-/-}$ tumors expressed low levels of MHC class I. Although *RAG2*$^{-/-}$ mice lack functional T cells, B cells, or NKT or $\gamma\delta$T cells, they have fully functional NK cells that mediate tumor surveillance in part through recognizing as nonself, and destroying, cells that express low levels of MHC class I. Evidence that supports an important role for MHC class I in these experiments includes the observation that restoration of MHC class I restored the leukemogenic potential of *v-abl*-transformed *STAT1*$^{-/-}$ cells. Furthermore, in those animals in which disease did develop, the *v-abl*-transformed *STAT1*$^{-/-}$ leukemic cells showed evidence of in vivo "immunoediting" (Shankaran et al., 2001) with apparent selection for leukemia cells that had acquired stable high-level expression of MHC class I.

Of interest, these investigators also assessed the potential role of STAT1 in NK cell function, based on previous reports that *STAT1*$^{-/-}$ NK cells had reduced cytotoxic activity (Lee et al., 2000a). They demonstrated that *STAT1*$^{-/-}$ NK cells, despite reduced cytotoxic activity, could kill *v-abl*-transformed *STAT1*$^{-/-}$, but not v-abl-transformed *STAT1*$^{+/-}$, leukemia cells. In consonance with these in vitro findings, there was an attenuated, but not abrogated, leukemia phenotype in STAT1$^{-/-}$ neonates transduced with v-abl retrovirus, with 6/28 animals remaining healthy for more than 8 months, whereas all *STAT1*$^{+/-}$ animals succumbed to disease. Furthermore, among the 22 *STAT1*$^{-/-}$ animals that did develop disease, there was increased MHC class I expression on leukemic cells, consistent with an immunoediting activity in vivo. It is important as well to note that the observed defects in *STAT1*$^{-/-}$ mice are quite different than those observed with *IFN-*$\gamma^{-/-}$ mice, suggesting that aberrancies in the *STAT1*$^{-/-}$ context may be due in part to IFN-independent mechanisms (Lee et al., 2000b).

Another interesting point that can be gleaned from the cumulative data is the very delicate balance between cell-autonomous and -nonautonomous contributions to phenotype—referred to by the authors as an "evolutionary arms race" in tumorigenesis. These data derived from transplantation into *RAG2*$^{-/-}$ mice provide additional support for the importance of innate immunity mediated by NK cells, in addition to involvement of acquired tumor immunity mediated by T and B cells. It would be of interest to test these findings in mice deficient in NK cells (such as *FLT3*$^{-/-}$-deficient animals) to obtain insights into the relative contributions of innate versus acquired immunity in this model.

The delicate balance in tumorigenic potential versus cell-nonautonomous tumor surveillance is exemplified in several experiments. For example, *v-abl*-transformed *STAT1*$^{-/-}$ cells show an attenuated phenotype, presumably due to decreased expression of MHC class I. But this finding is dependent on use of low cell doses, and in instances where leukemia develops the *STAT1*$^{-/-}$ leukemia cells have been "immunoedited" by NK cells to include only those that now express high levels of MHC class I. Furthermore, although *STAT1*$^{-/-}$ NK cells are defective in cytotoxic activity, they can still attenuate leukemic phenotypes engendered by *STAT1*$^{-/-}$ leukemic cells. In contrast, fully competent *STAT*$^{+/-}$ NK cells could efficiently eradicate *STAT1*$^{-/-}$ leukemic cells, but not *STAT1*$^{+/-}$ cells that express high levels of MHC class 1. These observations provide some insight into the complexities and challenges—and thus far limited success—of manipulating host immunity to improve outcome in treatment of human cancers. Tumor burden is clearly an important issue, as are host determinants of both innate and humoral immunity. Murine models, such as the one described here, should enable detailed study of this complex interplay.

It is not yet clear whether the findings in this model system can be broadly extrapolated, or the extent to which these mouse models of immune surveillance might inform therapeutic strategies in humans. The authors suggest that loss of STAT1 may be relevant in other contexts, including leukemia initiated by v-abl-transformed *STAT1*$^{-/-}$ cells in immunocompetent hosts such as Balb/C. However, the *v-abl* allele is not a known cause of hematological malignancies in humans. Another activated *ABL* allele, *BCR-ABL*, is associated with chronic myelogenous leukemia (CML) in humans. But, CML is a special case among hematopoietic malignancies in being highly responsive to IFN therapy. Thus, it will be important to test other leukemogenic alleles in addition to activated *ABL*. Limited analysis of the rare *TEL-JAK2* allele is provided but focuses on erythroid progenitors that are not a feature of the spectrum of *TEL-JAK2* leukemias in humans, and curiously shows significant attenuation of phenotype in a *RAG2*$^{-/-}$ $\gamma C^{-/-}$ background compared to Balb/C, though no difference was observed in that background between *STAT*$^{+/-}$ and *STAT1*$^{-/-}$ leukemic cells. Additional analysis will be necessary to determine to what extent these phenomena can be extrapolated to several of the more common human hematological malignancies.

However, there are several key points that emanate from this interesting report. These findings serve as an important extension of the concept of immunoediting in tumor surveillance (Shankaran et al., 2001), and the importance of NK cells in shaping the phenotypic characteristics of tumors. These data corroborate and support previous findings on the role of STAT1 in NK cell function, and in regulation of MHC class I expression. It seems likely, based on these data, that there will be functions of STAT1 that are both dependent and independent of type I IFN signaling, an arena that should be of considerable interest for further investigation. These experiments also elegantly demonstrate the value of modeling cancer in vivo in murine models of disease, and the importance of in vivo experimentation to dissect out the evolutionary "winner" in cell-autonomous tumor-promoting activity, in apposition to cell-nonautonomous host immune response—in this case each attributable to deficiency of the same allele, *STAT1*. Finally, this report demonstrates an expanding paradigm that some gene products may serve both as tumor suppressors and as tumor promoters. Ultimately, clear insights into these pathogenetic processes should inform more effective therapeutic approaches to cancer.

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Selected reading

Kovacic, B., Stoiber, D., Moriggl, R., Weisz, E., Ott, R.G., Kreibich, R., Levy, D.E., Beug, H., Freissmuth, M., and Sexl, V. (2006). Cancer Cell, this issue.

Lee, C.K., Gimeno, R., and Levy, D.E. (1999). J. Exp. Med. *190*, 1451–1464.

Lee, C.K., Rao, D.T., Gertner, R., Gimeno, R., Frey, A.B., and Levy, D.E. (2000a). J. Immunol. *165*, 3571–3577.

Lee, C.K., Smith, E., Gimeno, R., Gertner, R., and Levy, D.E. (2000b). J. Immunol. *164*, 1286–1292.

Lesinski, G.B., Anghelina, M., Zimmerer, J., Bakalakos, T., Badgwell, B., Parihar, R., Hu, Y., Becknell, B., Abood, G., Chaudhury, A.R., et al. (2003). J. Clin. Invest. *112*, 170–180.

Shankaran, V., Ikeda, H., Bruce, A.T., White, J.M., Swanson, P.E., Old, L.J., and Schreiber, R.D. (2001). Nature *410*, 1107–1111.

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Of mice and men: Cancer gene discovery using comparative oncogenomics

With the proliferation of high-throughput technologies to profile the cancer genome, methods to distinguish causal from bystander genetic events are needed. Two recent reports by Zender et al. and Kim et al. in *Cell* use genetically defined mouse models to serve as biological filters to mine the human cancer genome. Integration of high-resolution copy number profiles of mouse tumor models and human tumors identified *cIAP1* and *Yap* as oncogenes in human hepatocellular carcinoma, while *NEDD9* was identified as a metastasis gene in human melanoma. Together, these reports demonstrate that a comparative oncogenomics approach can identify genes causally involved in oncogenesis and metastasis.

High-throughput techniques are identifying remarkable heterogeneity in the cancer genome, implying that dysregulation of numerous genes and pathways can lead to oncogenesis and cancer progression. Furthermore, as cancers progress, they demonstrate genomic instability, leading not only to the acquisition of mutations conferring selective advantages, but also to noncontributing bystander lesions. With plans to characterize all mutations in human cancers, such as The Cancer Genome Atlas (Bonetta, 2005), novel techniques to identify the lesions driving oncogenesis/metastasis from secondary mutations are urgently needed. Two studies by Zender et al. (2006) and Kim et al. (2006) in the June 30 issue of *Cell* demonstrate the usefulness of comparative oncogenomic approaches using defined mouse models to identify driving genes in oncogenesis and metastasis in human tumors (Figure 1).

Comparative oncogenomics identifies* cIAP1 *and* Yap *as driving oncogenes in hepatocellular carcinoma

In the study by Zender et al., the authors isolated hepatoblasts from the liver of *p53*$^{-/-}$ mice and overexpressed *Myc*, activated *Akt*, or oncogenic *Ras* before reintroducing these cells into recipient mice livers. They focused on tumors developing from *p53*$^{-/-}$;*myc*-induced hepatoblasts, which were histologically consistent with human hepatocellular carcinomas (HCCs). In an effort to identify spontaneously acquired lesions contributing to tumorigenesis, they used a form of array comparative genomic hybridization (aCGH) termed representational oligonucleotide microarray analysis (ROMA) to search for focal regions of copy number change. By ROMA, four of seven *Myc*-driven HCCs contained a focal amplicon (containing 12 genes) on chromosome 9qA1.

Using the mouse model as a biological filter, the authors interrogated 48 human HCCs profiled in parallel. Intriguingly, two of these human HCCs harbored focal amplifications on chromosome 11q22 (syntenic to mouse 9qA1). To identify the driving gene, the authors determined the expression of all overlapping genes from the syntenic amplicons. Not one, but two genes, *cIAP1* and *Yap*, showed increased expression at the mRNA and protein level in all mouse and human tumors containing the amplicon.

To confirm the oncogenic effects of *cIAP1* and *Yap* in vivo, the authors overexpressed these two genes in the *p53*$^{-/-}$;*myc* hepatoblasts that were used to generate the tumors. Expression of *cIAP1* or *Yap*