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.

DOI 10.1016/j.ccr.2006.06.015

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*

accelerated tumor formation and increased tumor burden. Similarly, knockdown of *cIAP1* or *Yap* in tumors containing the 9qA1 amplicon inhibited tumor growth. Finally, the authors demonstrated that *cIAP1* and *Yap* act synergistically in the 9qA1 amplicon, as coexpression of *cIAP1* and *Yap* accelerated tumor growth more than expression of either gene alone.

Comparative oncogenomics identifies NEDD9 as a mediator of metastasis in melanoma

Kim et al. employed a similar approach, using an inducible *Ras* model of nonmetastatic melanoma to generate "escapers," or clones that underwent secondary genomic events conferring metastatic capability. The authors characterized two such escapers with increased proliferation and invasion compared to the parental tumors and profiled these samples using genome-wide aCGH. Both escapers showed a focal amplification on mouse chromosome 13, and the overlapping region contained eight genes. Examining the expression of these eight genes in *Ras*- and *Met*-driven melanomas, *NEDD9* was the only gene with increased expression in samples with and without genomic amplification.

The authors used this result to guide the examination of a database of human aCGH profiles from melanocytic lesions, where chromosome 6p25-24 (syntenic to mouse chromosome 13) was found to be amplified in 36% of metastatic melanomas, 8% of melanomas, and none of the benign nevi. They confirmed the overexpression of *NEDD9* at the transcript and protein levels in 35%–52% of metastatic melanomas using quantitative RT-PCR and immunohistochemistry on tissue microarrays. To confirm the role of *NEDD9* in the development of metastasis in their escapers, the authors knocked down *NEDD9* expression, significantly inhibiting the proliferation and invasion of the two escapers. Additionally, the authors demonstrated that overexpression of *NEDD9* increased the in vitro and in vivo metastatic capability of primary melanocytes and nonmetastatic melanoma cells.

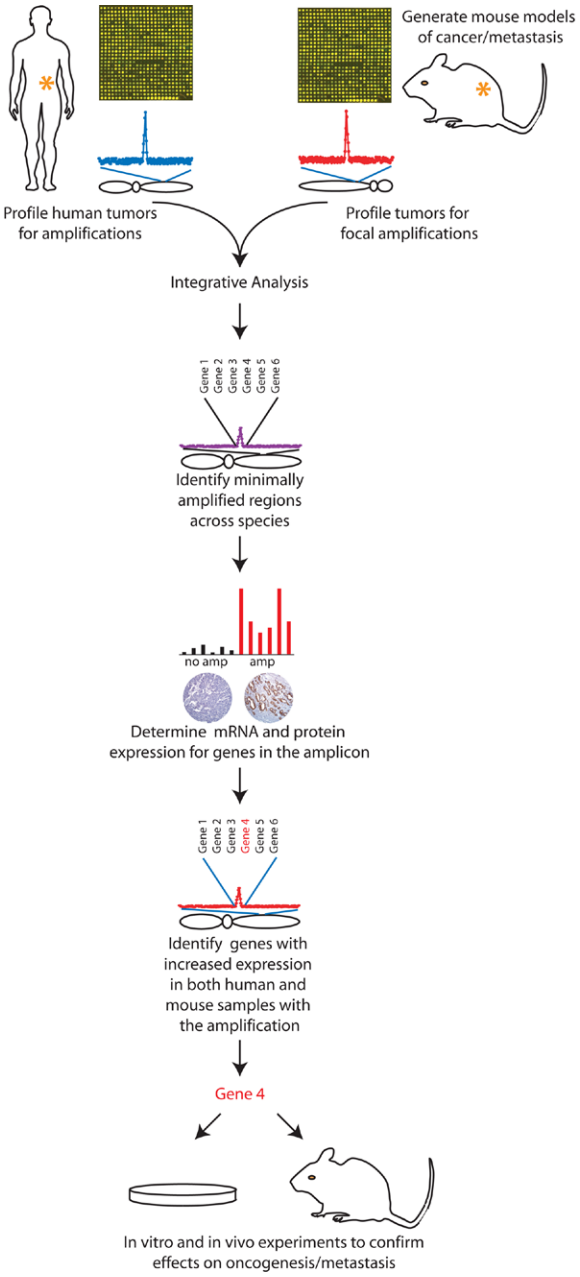

Figure 1. Biological filtering using defined mouse models to identify driving mutations controlling oncogenesis/metastasis in human tumors

Schematic of the experimental approach used by Zender et al. and Kim et al. to identify genes involved in tumor initiation or metastasis. Defined mouse models of tumorigenesis/metastasis were chosen to mimic the initiation or progression of hepatocellular carcinoma or melanoma, respectively. Isolated tumors from these models were profiled by array comparative genomic hybridization (aCGH)-based techniques to identify focal regions of copy number change. These profiles were then used to filter aCGH data from human tumors to identify minimally conserved amplified regions between the mouse and human tumors. The expression of all genes in the amplified regions in both the mouse models and human tumor samples was determined. Candidate gene(s) were chosen by increased expression in all mouse and human samples with the amplification. Importantly, both groups were then able to confirm the effect of their candidate gene(s) in vitro or in vivo in the same defined genetic model by retroviral-mediated overexpression or shRNA knockdown.

Finally, the authors showed that *NEDD9* expression induced the activation of FAK, a key regulator of focal adhesion contacts, and demonstrated that NEDD9 modulates the formation of focal contacts in melanocytes, suggesting a mechanism for its effects on invasion and metastasis.

Advantages and limitations of mouse models to identify driving cancer genes

An important aspect of both studies was the use of mouse models with defined genetic backgrounds. This provides the proper genetic background to confirm the oncogenic/metastatic effects of identified candidate genes, as these effects may only occur in cooperation with the lesions used to generate the tumor models. For example, Zender et al. demonstrated that *cIAP1* and *Yap* overexpression did not affect tumor formation in *p53*$^{-/-}$;*Akt* or *Ras* hepatoblasts. Likewise, Kim et al. showed that *NEDD9* was unable to increase invasion or metastasis in melanocytes in the absence of activated *Ras* or *Raf*. Unfortunately, for many tumor types, the initiating genetic events driving tumorigenesis and the precursor cells harboring such events are unclear, precluding the development of such defined models. In addition, the experimental strategies utilized by Zender et al. and Kim et al., which were designed to identify focal amplifications driving oncogenesis and metastasis, would not be able to identify driving mutations or chromosomal rearrangements.

Integrative approaches to identify genes causally involved in cancer

Another important result observed by both Zender et al. and Kim et al. was that the oncogenic amplifications that they observed in human tumors were relatively rare. For example, Zender et al. observed amplification of the *cIAP1* and *Yap* loci in only 2 of 48 (4%) human hepatocellular carcinomas, while Kim et al. observed amplification of the region harboring *NEDD9* in 36% of metastatic melanomas. These results likely reflect the substantial genetic heterogene-

ity that underlies tumorigenesis and the development of metastasis. Recently, our group developed a bioinformatics strategy termed Cancer Outlier Profile Analysis (COPA) in an effort to identify "oncogene outliers," or genes with marked overexpression in a fraction of cases, which characterize genes involved in high-level copy number changes or translocations, such as those described above. COPA identified the *Ets* transcription factors *ERG* and *ETV1* as outliers across multiple prostate cancer profiling studies. Characterizing cases with overexpression of *ERG* or *ETV1*, we demonstrated that these samples harbored recurrent gene fusions with the androgen-regulated gene *TMPRSS2*, and these fusions occur in the majority of prostate cancers (Tomlins et al., 2005). This approach was useful in the analysis of tumors without biologically relevant mouse models and may prove useful in mining mouse models of cancer as well.

In addition to using mouse models or bioinformatic strategies, other groups have used alternative integrative analyses to identify driving genetic events in tumorigenesis. For example, Garraway et al. performed an integrative analysis combining gene expression and copy number profiles of the NCI60 panel of cancer cell lines to identify and validate *MITF* as a lineage-specific oncogene amplified in a subset of melanomas (Garraway et al., 2005). Adler et al. also integrated expression and copy number profiles from breast cancers to identify coordinated amplification of *Myc* and *CSN5* as a regulator of the wound response profile that is predictive of poor outcome (Adler et al., 2006).

Amassing genomic scale data for human tumors is becoming relatively routine. The rapid advancement of high-throughput techniques suggests that complete genomic profiles of tumor samples may soon be realized. Integrative approaches will be needed to sift through this mass of data to identify the driving genetic lesions underlying cancer development and progression. The results of Zender et al. and Kim et al. demonstrate that integrative approaches based on defined mouse models can be used as one such method to identify driving oncogenic or metastatic events in human tumors.

Acknowledgments

This work was supported in part by the Department of Defense (W81XWH-06-1-0224), the National Institutes of Health (U54 DA021519-01A1 to A.M.C.), and the Cancer Center Bioinformatics Core (Support Grant 5P30 CA46592). S.A.T. is supported by a Rackham Predoctoral Fellowship and is a Fellow of the Medical Scientist Training Program. A.M.C. is supported by a Clinical Translational Research Award from the Burroughs Welcome Foundation.

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Selected reading

Adler, A.S., Lin, M., Horlings, H., Nuyten, D.S., van de Vijver, M.J., and Chang, H.Y. (2006). Nat. Genet. *38*, 421–430.

Bonetta, L. (2005). Cell *123*, 735–737.

Garraway, L.A., Widlund, H.R., Rubin, M.A., Getz, G., Berger, A.J., Ramaswamy, S., Beroukhim, R., Milner, D.A., Granter, S.R., Du, J., et al. (2005). Nature *436*, 117–122.

Kim, M., Gans, J.D., Nogueira, C., Wang, A., Paik, J.-H., Feng, B., Brennan, C., Hahn, W.C., Cordon-Cardo, C., Wagner, S.N., et al. (2006). Cell *125*, 1269–1281.

Tomlins, S.A., Rhodes, D.R., Perner, S., Dhanasekaran, S.M., Mehra, R., Sun, X.W., Varambally, S., Cao, X., Tchinda, J., Kuefer, R., et al. (2005). Science *310*, 644–648.

Zender, L., Spector, M.S., Xue, W., Flemming, P., Cordon-Cardo, C., Silke, J., Fan, S.-T., Luk, J.M., Wigler, M., Hannon, G.J., et al. (2006). Cell *125*, 1253–1267.

DOI 10.1016/j.ccr.2006.06.013

Differential utilization of two ATP-generating pathways is regulated by p53

A fundamental property of cancer cells is the preferential utilization of glycolysis over aerobic respiration to produce ATP. Renewed interest in understanding the mechanism underlying this metabolic shift in energy production is broadening our understanding of the relationship between cancer and cellular metabolism. In a recent article, Matoba et al. report that the p53 tumor suppressor regulates the expression of SCO2, a protein that is required for the assembly of cytochrome c oxidase (COX), a multimeric protein complex required for oxidative phosphorylation. The implication of these findings is that aerobic respiration is compromised in cells that lack functional p53.

In contrast to normal cells, cancer cells have a high glycolytic rate and produce high levels of lactate even in the presence of oxygen (Figure 1). This metabolic shift to a higher rate of aerobic glycolysis is commonly referred to as the Warburg effect. Because glycolysis produces energy (ATP) far less efficiently than aerobic respiration, tumor cells have a much higher rate of glucose uptake than normal cells (Figure 1). The physiological significance of the Warburg effect has been controversial since its discovery over 80 years ago, and now there is renewed and vigorous interest in understanding the relationship between cancer and altered energy metabolism. A commonly held view is that constitutive upregulation of glycolysis is likely to be an adaptation to hypoxia that develops as tumor cells grow progressively further away from their blood supply (Gatenby and Gillies, 2004). Interestingly, cells derived from tumors continue to utilize glycolysis in culture under normoxic