On the surface these studies appear to conflict with the work of Somervaille and Cleary, in that the immunophenotype of AML-SCs is quite different. However, it is actually not possible to directly compare the reports due to substantial differences in methodology. The two groups employed different populations of normal cells for the original transduction with MLL-AF9, different strategies for functional characterization of AML-SCs, and different methods to estimate the frequency of AML-SCs. Taken together, these factors could certainly lead to varying interpretations and conclusions, despite the common feature of studying MLL-AF9-induced leukemias.

Considered more broadly, the reports of Somervaille and Cleary (2006) and Krivtsov et al. (2006) serve to emphasize an intriguing point—the basic properties of AML-SCs may be relatively heterogeneous and may vary as a function of genetics and developmental origin. Figure 1 illustrates some of the hypothetical steps leading to the generation of AML-SCs. When one considers the specific type of initial mutation (step 1), the originating target cell (step 2), subsequent differentiation or lack thereof (step 3), and the types of subsequent mutations that may occur (step 4), the number of possible permutations in the genesis of AML-SCs is quite large. Furthermore, given the inherently unstable genome of most malignant cells and possible changes evoked by challenge with various drug therapies, the resulting phenotype of human AML-SCs is potentially even more complex.

Given the issues above, several questions regarding the evolution and properties of AML-SCs should be considered. Perhaps most importantly, while the elegant studies in mouse models have indicated differing paths by which leukemia stem cells may arise, are any of these scenarios prevalent in primary human disease? To date, the only direct studies indicating a GMP-like origin for an acute form of myeloid disease is the report by Jamieson et. al. that describes studies of blast crisis CML (chronic myeloid leukemia) (Jamieson et al., 2004). CML is unique among myeloid leukemias in that it displays an overt and well-defined pathology at each stage of progression, and thereby permits the isolation and analysis of stem cells from early (chronic) and late (blast crisis) forms of disease. However, aside from CML, in five of the other eight major subtypes of AML (designated as FAB types M0, M1, M2, M4, and M5), the only AML-SCs characterized to date are both rare and phenotypically similar to HSCs (Bonnet and Dick, 1997). Therefore, while the data from mouse models are compelling, their direct relevance to human disease remains a largely unanswered question. Indeed, from a genetic perspective, evidence suggests that transformation of human cells is more complex than murine cells (Rangarajan and Weinberg, 2003); thus, findings from one species should be validated in the other. Going forward, it will be important to identify and isolate human counterparts to the entities described thus far from murine studies. Such efforts may come from the analysis of primary specimens, as described by Jamieson et al., or may also derive from the generation of better experimental models. In this regard, the studies cited above using gene transfer into normal murine progenitor cells have likely established an important precedent for future efforts using primary human cells.

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Selected reading

Bonnet, D., and Dick, J.E. (1997). Nat. Med. *3*, 730–737.

Cozzio, A., Passegue, E., Ayton, P.M., Karsunky, H., Cleary, M.L., and Weissman, I.L. (2003). Genes Dev. *17*, 3029–3035.

Huntly, B.J., and Gilliland, D.G. (2005). Nat. Rev. Cancer *5*, 311–321.

Huntly, B.J., Shigematsu, H., Deguchi, K., Lee, B.H., Mizuno, S., Duclos, N., Rowan, R., Amaral, S., Curley, D., Williams, I.R., et al. (2004). Cancer Cell *6*, 587–596.

Jamieson, C.H., Ailles, L.E., Dylla, S.J., Muijtjens, M., Jones, C., Zehnder, J.L., Gotlib, J., Li, K., Manz, M.G., Keating, A., et al. (2004). N. Engl. J. Med. *351*, 657–667.

Krivtsov, A.V., Twomey, D., Feng, Z., Stubbs, M.C., Wang, Y., Faber, J., Levine, J.E., Wang, J., Hahn, W.C., Gilliland, D.G., et al. (2006). Nature *442*, 818–822.

Lapidot, T., Sirard, C., Vormoor, J., Murdoch, B., Hoang, T., Caceres-Cortes, J., Minden, M., Paterson, B., Caligiuri, M.A., and Dick, J.E. (1994). Nature *367*, 645–648.

Rangarajan, A., and Weinberg, R.A. (2003). Nat. Rev. Cancer *3*, 952–959.

Somervaille, T.C.P., and Cleary, M.L. (2006). Cancer Cell, this issue.

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AKT and cancer—Is it all mTOR?

AKT, a key regulator of cell proliferation and survival, is commonly dysregulated in human cancers. Activated AKT kinase is oncogenic and required for tumorigenesis in PTEN-deficient animals. However, the importance of AKT in mediating transformation by other oncogenes and which of its targets are necessary for this process are poorly understood. In this issue of *Cancer Cell*, Skeen et al. show that AKT is required for transformation by mutant H-Ras and for experimental skin carcinogenesis. Moreover, the effects of AKT are mediated predominantly or solely via mTORC1. This suggests that AKT or mTOR inhibitors will be useful treatments for many cancers.

The PI3K/AKT kinase pathway is a central regulator of cell metabolism, proliferation, and survival and is dysregulated by oncogenic events in a substantial fraction of tumors. Constitutive activation of growth factor receptors, mutation of PI3K, and inactivation of the PTEN phosphatase cause the activation of PI3K signaling in the majority of glioblastomas and breast, endometrial, and prostate cancers, among others. Furthermore, PI3K is an effector of RAS function and

has been shown to be required for both the development and maintenance of tumors driven by mutant H-Ras (Lim and Counter, 2005).

PI3K has a multitude of downstream targets, including AKT, mutations of which are oncogenic and occasionally present in human tumors. AKT activation stimulates proliferation and desensitizes cells to apoptotic stimuli. Recently, Hay and colleagues showed that AKT1 is required for the development of tumors in *Pten*$^{+/-}$ mice (Chen et al., 2006). This result proved that AKT is a target of PI3K that is necessary for transformation driven by PTEN haploinsufficiency. It left open the question of whether AKT was important in mediating transformation by other oncogenes, and through which targets AKT exerted those effects.

These issues are addressed in a follow-up paper in this issue of *Cancer Cell*. Skeen et al. (2006) show that the proliferation of mouse embryonic fibroblasts from Akt1 null mice is impaired, as is their sensitivity to transformation by H-Ras in combination with dominant-negative p53. They further demonstrate that tumorigenesis in mutant H-Ras transgenic mice and in a carcinogen-induced murine skin cancer model are markedly reduced in the Akt1 null background. Although PI3K is recognized as an important effector of activated Ras, these data strongly suggest that, at least in these contexts, PI3K activation of AKT is necessary for transformation and, perhaps, a reasonable target for therapy.

Next, Skeen et al. investigate whether mTORC1 is the downstream target of AKT that mediates its effects on transformation. AKT signals through a variety of downstream targets that affect transcription, metabolism, and apoptosis. Phosphorylation of TSC2 by AKT relieves the inhibition of Rheb/mTORC1 by the TSC1/2 complex and serves to activate translation via the effects of mTOR on p70S6 kinase and 4EBP1 (Ruggero and Sonenberg, 2005). Tsc2 is a tumor suppressor gene, germline mutations of which are associated with tuberous sclerosis. Inactivation of TSC2 causes tumors by constitutively activating mTOR. mTOR activation also inhibits AKT via an S6K-dependent feedback mechanism (Harrington et al., 2004; Um et al., 2004).

In Tsc2 null cells, therefore, mTORC1 is activated and AKT is inhibited. Skeen et al. show that these cells have normal rates of proliferation and are quite sensitive to transformation by H-Ras. In these cells, reducing Raptor expression with shRNA decreases mTOR activity and, by relieving feedback, increases AKT activity, yet sensitivity to H-Ras is reduced. Conversely, introducing activated Rheb into Akt1/2 double null cells restores their transformability. This work convincingly shows that, in this system, the AKT dependence of H-Ras transformation is mediated by mTORC1.

These findings have important implications. They suggest that other downstream targets of AKT are dispensable for its role in transformation by H-Ras. Furthermore, the work implies that drugs that cause even partial inhibition of AKT activity could be extremely useful for the treatment of many classes of tumors, including those with Ras, PI3K, and Pten mutations. Finally, Skeen et al. show that mTORC1 inhibition with rapamycin inhibits transformation by H-RAS. By relieving feedback inhibition, rapamycin causes increased AKT activity in some tumors (O'Reilly et al., 2006). This has raised the concern that relief of feedback inhibition could attenuate the effects of rapamycin or even cause tumors to worsen after treatment with the drug. However, if the only important downstream target of AKT is mTOR, this should not be a problem. The data in Skeen et al. support the idea that mTOR inhibitors should be useful in a broad range of tumors.

Skeen et al. studied the transformation of murine cells, mostly fibroblasts, with mutant H-Ras. It is worth pointing out some areas in which their conclusions diverge from those drawn from observations in other systems. Tsc2 mutations have not been detected in sporadic tumors. Tumors that arise in a Tsc2 mutant background, in humans and in model systems, are indolent and have reduced AKT signaling. However, PTEN haploinsufficiency restores AKT signaling and causes more rapidly growing, virulent tumors (Manning et al., 2005). These findings suggest that activation of mTOR alone is not sufficient to mediate AKT-dependent transformation in this system and that AKT must have other downstream targets.

It is possible that mTORC1 is the dominant AKT target in cells transformed with mutant Ras, but not in others. Several RAS effectors are necessary for its induction of transformation. RAS/RAF/MAPK signaling plays important roles in regulating transcription and apoptosis and may complement mTOR and render other AKT targets superfluous (Rajasekhar et al., 2003; She et al., 2005). However, whereas mutant H-Ras is quite transforming in murine systems and used ubiquitously in laboratory studies, it is rare in human tumors, in which K-Ras and N-Ras mutations dominate. It is very possible that mutant H-Ras has different properties than the others and may be less relevant to human systems.

Finally, this work and that of others strongly suggests the utility of both AKT and mTOR inhibitors in the treatment of human cancer. Previous studies suggest that tumors with mutational activation of PI3K or AKT or loss of PTEN function would be especially sensitive to mTOR inhibition (Neshat et al., 2001; Podsypanina et al., 2001). This work adds tumors with Ras mutation to the list. A large percentage of human tumors should therefore be sensitive to rapamycin-like drugs. Unfortunately, this has not proven to be the case. Three analogs of rapamycin that effectively inhibit the Raptor-mTOR complex have been tested extensively in patients with advanced cancer. These drugs have modest anticancer activity in a minority of patients, including some with renal cancer, sarcomas, and mantle cell lymphoma. However, results have been quite disappointing, especially in tumors characterized by a high frequency of Pten mutation (glioblastoma, prostate cancer) or K-Ras mutation (pancreatic and lung cancer). There are many possible reasons for resistance, including ineffective target inhibition and coexisting mutations that activate other signaling pathways. It has been suggested that the rapamycin-induced activation of AKT might attenuate its effects. In this case, combined therapy with mTOR and PI3K/AKT inhibitors should be useful. In support of this idea, Weiss et al. have shown that a drug that inhibits both mTOR and PI3K has enhanced activity in glioblastoma models (Fan et al., 2006).

One of the major goals of cancer biology is the development of rational clinical strategies based on an understanding of the biology of transformation. Clinical trials of specific inhibitors of key signaling proteins provide valuable information about the role played by the target in the tumor. The effects of imatinib in patients with chronic myelogenous leukemia are consistent with the central importance of BCR-ABL in this disease. The role played by mTOR in malignancy is clearly more complex. Just as data from model systems inform the development of clinical strate-

gies, analysis of laboratory data should take into account the results of clinical trials. The results of the clinical trials of rapamycin in cancer should be incorporated into our thinking about the role of mTOR signaling in transformation.

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Selected reading

Chen, M.L., Xu, P.Z., Peng, X.D., Chen, W.S., Guzman, G., Yang, X., Di Cristofano, A., Pandolfi, P.P., and Hay, N. (2006). Genes Dev. *20*, 1569–1574.

Fan, Q.W., Knight, Z.A., Goldenberg, D.D., Yu, W., Mostov, K.E., Stokoe, D., Shokat, K.M., and Weiss, W.A. (2006). Cancer Cell *9*, 341–349.

Harrington, L.S., Findlay, G.M., Gray, A., Tolkacheva, T., Wigfield, S., Rebholz, H., Barnett, J., Leslie, N.R., Cheng, S., Shepherd, P.R., et al. (2004). J. Cell Biol. *166*, 213–223.

Lim, K.H., and Counter, C.M. (2005). Cancer Cell *8*, 381–392.

Manning, B.D., Logsdon, M.N., Lipovsky, A.I., Abbott, D., Kwiatkowski, D.J., and Cantley, L.C. (2005). Genes Dev. *19*, 1773–1778.

Neshat, M.S., Mellinghoff, I.K., Tran, C., Stiles, B., Thomas, G., Petersen, R., Frost, P., Gibbons, J.J., Wu, H., and Sawyers, C.L. (2001). Proc. Natl. Acad. Sci. USA *98*, 10314–10319.

O'Reilly, K.E., Rojo, F., She, Q.B., Solit, D., Mills, G.B., Smith, D., Lane, H., Hofmann, F., Hicklin, D.J., Ludwig, D.L., et al. (2006). Cancer Res. *66*, 1500–1508.

Podsypanina, K., Lee, R.T., Politis, C., Hennessy, I., Crane, A., Puc, J., Neshat, M., Wang, H., Yang, L., Gibbons, J., et al. (2001). Proc. Natl. Acad. Sci. USA *98*, 10320–10325.

Rajasekhar, V.K., Viale, A., Socci, N.D., Wiedmann, M., Hu, X., and Holland, E.C. (2003). Mol. Cell *12*, 889–901.

Ruggero, D., and Sonenberg, N. (2005). Oncogene *24*, 7426–7434.

She, Q.B., Solit, D.B., Ye, Q., O'Reilly, K.E., Lobo, J., and Rosen, N. (2005). Cancer Cell *8*, 287–297.

Skeen, J.E., Bhaskar, P.T., Chen, C.-C., Chen, W.S., Peng, X.-d., Nogueira, V., Hahn-Windgassen, A., Kiyokawa, H., and Hay, N. (2006). Cancer Cell, this issue.

Um, S.H., Frigerio, F., Watanabe, M., Picard, F., Joaquin, M., Sticker, M., Fumagalli, S., Allegrini, P.R., Kozma, S.C., Auwerx, J., and Thomas, G. (2004). Nature *431*, 200–205.

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