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A knotty turnabout?: Akt1 as a metastasis suppressor

Akt is well known to enhance malignancy and is recognized as a key target for antineoplastic therapies. However, intriguing findings reported by Yoeli-Lerner et al. in the November 23, 2005 issue of *Molecular Cell*, suggest a novel, antimetastasis function of Akt: activation of Akt1 inhibited invasion in some cancer cells. One possible mechanism for this surprising phenotype was that Akt activated the E3 ubiquitin ligase HDM2, causing ubiquitination and degradation of NFAT, an invasion-promoting factor. These findings clearly justify further investigations and, if validated in vivo, call for reevaluation of some Akt-targeting therapeutic strategies currently under development.

Akt modulates a variety of cellular processes, including proliferation, growth, and survival, by phosphorylating target proteins involved in these processes (recently reviewed in Bellacosa et al., 2004; Brazil et al., 2004; Osaki et al., 2004; Woodgett, 2005). Many signaling pathways activate Akt through phosphatidylinositol-3-kinase (PI3K). PI3K converts phosphatidylinositol-4,5-bis-phosphate (PIP2) to phosphatidylinositol-3,4,5-triphosphate (PIP3). PIP3 recruits Akt to the membrane where Akt is activated. PTEN dephosphorylates PIP3 and antagonizes PI3K function. Additionally, several molecules, such as CTMP, TRB3, and PHLPP, bind to and directly regulate Akt (Brazil et al., 2004; Gao et al., 2005).

Well-characterized substrates of Akt include antiapoptotic proteins, such as FOXO, BAD, and IKK-β; cell cycle regulators, such as $p27^{kip1}$, $p21^{cip1}$, MDM2, and Myt1; and GSK-3, which is involved in a variety of processes (Brazil et al., 2004). Akt has also been shown to stimulate angiogenesis via regulation of eNOS and increase cell metabolism through the mTOR/p70S6K pathway (Luo et al., 2003). Additionally, Akt stimulates MMP secretion, activates the small GTPase Rac, and promotes epithelial-to- mesenchymal transition (EMT), three attributes that can lead to increased metastatic potential (Bellacosa et al., 2004; Luo et al., 2003; Zhou et al., 2004). In light of Akt's involvement in all these pathways, it is not surprising that Akt protein levels, enzymatic activities, and even gene copy numbers are increased in many different types of tumors. Akt upregulation has been seen in carcinomas of the prostate, breast, ovary, pancreas, colon, stomach, and thyroid (Luo et al., 2003; Osaki et al., 2004). The importance of increased Akt activity in tumorigenesis is further underscored by the identification of gain-of-function mutations in PI3K and loss of expression of the PTEN tumor suppressor gene in many tumors, which positively and negatively regulate Akt activation, respectively (Luo et al., 2003; Osaki et al., 2004; Saal et al., 2005).

Given these well-established mechanisms by which Akt activation promotes transformation, a publication by the Toker laboratory in the November 23, 2005 issue of *Molecular Cell* suggests a surprising twist on Akt's role in the tumorigenic process (Yoeli-Lerner et al., 2005). Compelling in vitro evidence indicates that Akt blocked motility and invasion, important metastasis-related properties, in three different breast cancer cell lines. This was studied by artificially activating Akt1 and also by activating Akt with IGF-1 as a physiological stimulus. Conversely, lowering Akt1 levels with siRNA restored, and in some cases enhanced, the invasive properties of the cells. The kinase activity of Akt was

required. The authors further investigated the mechanism of this Akt-mediated effect with a candidate gene approach. Using activated constructs and siRNA, they demonstrate the importance of two molecules, NFAT and HDM2, in invasion suppression by Akt. The Toker laboratory had previously discovered that the transcription factor NFAT (nuclear factor of activated T cells) is downstream of $\alpha_6\beta_4$ integrin and promotes carcinoma invasion (Jauliac et al., 2002). In the current study, they demonstrate that Akt downregulates the transcriptional activity of NFAT by drastically reducing NFAT protein levels. Akt activation led to NFAT ubiquitination by HDM2 and targeted NFAT for degradation by the proteasome. HDM2, the human homolog of MDM2 (murine double minute 2), is an E3 ubiquitin ligase and is regulated by Akt (Ashcroft et al., 2002; Zhou et al., 2001). When HDM2 levels were lowered using siRNA, the same invasion-promoting effect occurred as when Akt levels were reduced. Furthermore, the phenotype of cells with constitutively active Akt was partially reversed when HDM2 levels were reduced. Thus, HDM2 is downstream of Akt in mediating inhibition of invasive behavior (Yoeli-Lerner et al., 2005).

These unexpected results may be important clinically if they hold when tested in other systems, particularly in vivo. While in vitro assays provide standard indicators of motility and invasiveness, strong statements cannot be made about metastatic behavior without studying the process in a whole animal. Nevertheless, the current findings are in agreement with published work (Hutchinson et al., 2004) from the Muller lab using transgenic mouse models. In this analysis, bitransgenic mice with activated ErbB2 and activated Akt1, both driven by the mouse mammary tumor virus (MMTV) promoter, were generated. The bitransgenic mice developed mammary tumors with significantly shorter latency than mice with only activated ErbB2, and the bitransgenic tumors were more proliferative than their ErbB2-induced counterparts. Consistent with the findings in the current report, the bitransgenic mice with both activated Akt1 and ErbB2 had dramatically less lung metastasis from the mammary tumors than mice with activated ErbB2 alone (7% and 68%, respectively). However, the bitransgenic tumors were also more differentiated than the ErbB2-induced tumors. The inverse relationship between differentiation and metastasis is well established; therefore, it is unclear whether the decrease in metastasis in the bitransgenic mice was a direct effect of Akt signaling or a consequence of the differentiation state of the tumors.

Akt-mediated inhibition of metastatic properties is clearly cell type dependent. Akt1 enhances the motility and invasion of other cell lines, including HT1080 fibrosarcoma cells. The in vivo study (Hutchinson et al., 2004) from the Muller laboratory does not rule out the possibility of a limited, cell type-specific effect because transgene expression is restricted to specific cells within the mammary gland. More interesting is the authors' suggestion that different Akt isoforms (Akt1 versus Akt2) may modulate metastasis in opposing manners. Some isoform-specific roles have previously been demonstrated for the three Akt proteins (Akt1, Akt2, and Akt3; also named protein kinase B α, β, and γ); however, there is no clear evidence that the proteins have different substrate specificities. The different activities of the three Akts may arise from differences in their relative abundance in a given cell type, differential regulation once activated, or the capacity to be activated by different stimuli (Bellacosa et al., 2004; Brazil et al., 2004; Osaki et al., 2004). In the current study, activated Akt1 was ectopically expressed, and the siRNA downregulated endogenous Akt1 without affecting Akt2 levels (Yoeli-Lerner et al., 2005). In studies previously published by the Slamon laboratory, Akt2 increased invasive behavior in culture and metastasis in vivo of a number of breast and ovarian cancer cell lines (Arboleda et al., 2003). Notably, the MDA-MB-231 cell line was tested in both studies and provides a direct comparison between the functions of Akt1 and Akt2 in determining metastatic potential. Thus, an understanding of how the relative levels of Akt1 and Akt2 influence motility, invasion, and metastasis is a necessary avenue for future investigation. Collectively, these findings suggest that clinically applicable, isoform-specific Akt inhibitors need to be developed.

The current paper nicely shows the involvement of NFAT and HDM2 in Akt1 inhibition of invasive behavior in breast cancer cell lines. However, it is unlikely that this is the whole story. Akt typically phosphorylates multiple molecules to regulate a single biological process. In light of this general theme of Akt action, further investigation of the mechanisms used by Akt proteins to modulate the metastatic properties of transformed cells is necessary. Additionally, HDM2 ubiquitinates other proteins, including p53, which inhibits malignancy (Ashcroft et al., 2002; Zhou et al., 2001). Therefore, the effects of Akt modulation of HDM2 may be very complicated. Nonbiased approaches may be the most informative way to elucidate the mechanisms and functions of Akt and HDM2 in these cells. Furthermore, it will be interesting to know if different stimuli upstream of Akt have distinct effects on Akt-mediated invasive properties.

If validated, the current findings could have important clinical implications. Inhibiting the Akt and PI3K pathway might be a double-edged sword that blocks tumorigenesis but promotes invasion. Therefore, it is imperative that the molecular mechanism be thoroughly understood in order to develop a molecular profile predictive of patient response to PI3K/Akt pathway inhibitors, thereby allowing precisely tailored cancer therapies.

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Revealing the genomic heterogeneity of melanoma

The melanoma genome possesses numerous recurrent chromosomal rearrangements, and embedded within this complexity are clues critical to disease pathogenesis and response to therapy. High-resolution genome-wide DNA copy number approaches, in conjunction with gene-specific mutational analyses, appear poised to define keystone molecular events, provide more accurate classification schemes, and set the stage for the design of rational therapies that may finally have an impact on survival of this deadly disease.

The rapid rise in melanoma incidence and the high lethality associated with advanced disease (reviewed in Thompson et al., 2005) has motivated efforts to define the genetic and environmental factors driving melanoma genesis and progression. It is generally accepted that melanoma risk is modulated by skin pigmentation patterns, such as those linked to *MC1R* polymorphisms (Palmer et al., 2000), and early exposure to ultraviolet (UV) light (reviewed in Thompson et al., 2005). Stereotypical genetic lesions in melanoma include disruption of the *CDKN2A* familial melanoma locus that encodes for INK4A and ARF; activation of MAPK pathway components, commonly at the levels of BRAF and NRAS; and activation of the PI3K-AKT pathway through loss of PTEN (reviewed in Chudnovsky et al., 2005; Gray-Schopfer et al., 2005). Beyond these well-known and validated genetic events, genome-wide high-resolution technologies have been used to scan the highly complex melanoma genome, revealing the existence of additional genetic elements governing disease genesis and progression (Garraway et al., 2005; Curtin et al., 2005; O.K. and L.C., unpublished data). These data show that the life history of melanoma is shaped by extensive chromosomal rearrangements, particularly recurrent chromosomal gains/amplifications and losses/deletions. That these copy number alterations carry pathogenetic significance has been substantiated in a recent integrated genomics approach that has identified *MITF* as a lineage survival oncogene amplified in melanoma (Garraway et al., 2005). With increasing resolution of array CGH platforms for mapping chromosomal alterations and advances in expression and sequencing technologies, it is anticipated that the discovery of novel melanoma relevant genes will accelerate dramatically in the near future.

High-resolution charting of recurrent copy number aberrations by array CGH will also provide the basis for molecular classification that, when combined with clinical information, will define genotype-phenotype correlation and biomarkers that can enhance existing staging systems for patient stratification. For example, correlating melanomas arising in different anatomical sites with different UV exposure patterns to distinct genomic signatures should lead to an understanding of the genetic modulators and targets of UV's mutagenic actions in this cancer. In the November 17 issue of *The New England Journal of Medicine*, Curtin et al. (2005) took an important first step in defining such gene-environment interactions in melanoma.

In this study, Curtin and colleagues conducted a genome-wide analysis of DNA copy number and mutational analysis of *BRAF* and *NRAS* in 126 melanomas from individuals with varying UV exposure histories (Curtin et al., 2005). Distinctive patterns of genomic alterations as well as differences in frequencies of *BRAF* and *NRAS* mutations were observed among the four groups of melanomas examined. Genomic instability was most prominent in melanomas arising on skin protected from direct UV light. Specifically, acral melanomas of the palms and soles and mucosal melanomas exhibited high numbers of whole-genome gains and losses, intrachromosomal copy number changes, and focal amplifications (Figure 1). On the other hand, amplifications and deletions were infrequent in melanomas arising on skin with chronic sun-induced damage (as defined by evidence of solar elastosis on histology) and those from skin with intermittent UV exposure but without chronic damage (Figure 1). There were differences not only in the levels of genomic instability for the four melanoma subgroups, but also in their patterns of chromosomal gains and losses (Figure 1). Genomic classification was able to classify acral and mucosal melanomas with 89% accuracy. It was also possible to distinguish between melanomas from skin exhibiting signs of chronic sun-induced damage and those from skin without signs of damage with 84% accuracy. That genomic signatures capable of classifying melanoma from different anatomic sites can be defined is a definitive proof that melanoma is a genetically heterogeneous disease.

In addition to their genomic patterns, the mutational spectrum was different between melanomas from sun-exposed and sun-protected skin (Figure 1). In particular, this study extended the group's earlier findings that melanomas from chronically sun-exposed and non-sun-exposed skin differed significantly in the mutation frequency of *BRAF* (Maldonado et al., 2003). In all melanoma groups, *BRAF* and *NRAS* mutations were found to be mutually exclusive. The presence of activating *BRAF* mutations inversely correlated with copy number gains of *CCND1*, and both events were associated with higher levels of CCND1 protein expression. Amplification of *CDK4*, encoding a CCND1 binding partner, was commonly seen in