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# All Roads Lead to IKK $\epsilon$

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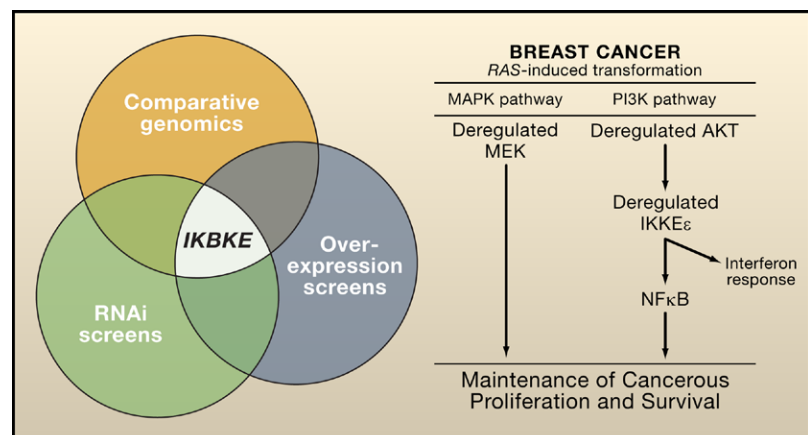
DOI 10.1016/j.cell.200.05.048

Extensive genetic alterations have been found in many epithelial malignancies. Using three complementary genetic approaches, Boehm et al. (2007) identify *IKBKE*—which encodes IKK $\epsilon$ , a component of the NF- $\kappa$ B pathway—as a breast cancer oncogene.

During malignant transformation, cancer cells acquire genetic alterations that surmount the normal controls regulating proliferation and cell survival. High-resolution comparative genomic analyses reveal that genomic instability is a hallmark of cancer development and that cancer-causing mutations are only a tiny fraction of the genetic modifications, most of which are “bystander” mutations. Thus, the discovery of the genetic alterations that contribute to tumor formation is crucial to the rational design of cancer therapeutics. But how can we distinguish cancer-causing mutations from other more benign mutations, and how do we identify genes whose activities either promote tumor development or protect us from cancer? In a report in this issue, Boehm et al. (2007) combine two functional genetic screens together with data from comparative genomic analyses to identify new protein kinases that promote tumor formation (Figure 1). These

efforts have led to the identification of a new oncogene in breast cancer, *IKBKE* (I-kappa-B kinase epsilon), which encodes IKK $\epsilon$ , an upstream regulator of the transcription factor NF- $\kappa$ B.

In the first genetic screen conducted by Boehm et al. (2007), a library of activated protein kinases was used in a cellular transformation system to recapitulate aspects of neoplastic transformation in vivo



**Figure 1. *IKBKE* Is a Breast Cancer Oncogene**

(Left) Identification of the *IKBKE* gene using three different genetic approaches. (Right) The RAS oncogenic pathway in breast cancer. Ras-induced transformation requires deregulation of the MAPK and PI3K signaling pathways. The *IKBKE* gene product, IKK $\epsilon$ , acts downstream of the dysregulated AKT kinase signaling pathway. By activating the NF- $\kappa$ B pathway through induction of nuclear accumulation of cRel, IKK $\epsilon$ , together with dysregulated MAPK signaling, induces tumor growth.

(Hahn et al., 1999). In this system, SV40 small t antigen and oncogenic *RAS* genes are overexpressed in primary human cells to stimulate tumorigenesis, and the p53 and the p16/pRb pathways are inhibited to prevent DNA-replication-damage-induced cellular senescence (Bartkova et al., 2006; Di Micco et al., 2006). Previous studies have shown that new oncogenes and tumor suppressor genes can be identified using primary human cells genetically designed to be one step short of full-blown transformation (Kolfchoten et al., 2005; Peeper et al., 2002; Voorhoeve et al., 2006; Westbrook et al., 2005). In the current study, Boehm and colleagues dissected the downstream pathways sufficient for *RAS* to induce neoplastic transformation. Although *RAS* can stimulate many pathways, such as the MAPK, PI3K, and RALGDS signaling pathways, a full-blown transformation phenotype of immortalized human cells could be acquired only by the simultaneous introduction of activated MEK (a kinase downstream of MAPK) together with a deregulated (myristoylated) AKT (a kinase downstream of PI3K; Figure 1). This observation presented the authors with an opportunity to screen a myristoylated kinase expression library for kinases that act in the place of AKT in the *RAS*-AKT pathway to promote cellular transformation. By doing this, the authors were able to identify the *IKBKE* gene and three other genes whose overexpression induced tumorigenesis.

In their second screen, Boehm et al. (2007) used an RNAi library designed to target 1200 kinases to find genes whose inactivation compromises cell growth and survival of a transformed (i.e., anchorage-independent) human breast cancer cell line but not the growth of an immortalized nontransformed cell line. Several RNAi constructs were identified, and among them three (out of five) constructs target the *IKBKE* gene. Blocking *IKBKE* expression through RNAi impeded the proliferation and survival of transformed

MCF-7 cells but not of immortalized nontransformed MCF-10A cells.

These two functional genetic approaches yielded several candidate oncogenes. To identify the genes among those candidates that not only alter normal cellular behavior but also are actively involved in human breast cancer development, Boehm et al. (2007) performed a comparative genomic analysis on a number of breast cancer cell lines and tumor specimens from cancer patients. Interestingly, *IKBKE* was the only candidate gene found to reside in a genomic region (1q32) that was amplified in many of the tumors analyzed, producing high expression levels of *IKBKE* RNA and protein. Furthermore, in many other tumor specimens, elevated expression of *IKBKE* was detected without being associated with gene amplification, indicating that several mechanisms can upregulate *IKBKE* expression. Yet mutations in *IKBKE* were not found, suggesting that *IKBKE* overexpression is more potent than activating mutations. Alternatively, it is also possible that mechanisms increasing *IKBKE* expression have greater effects on tumorigenesis than mutations or that there is a collaborating oncogene in the vicinity of *IKBKE*. Nonetheless, further investigation confirmed that *IKBKE* acts as an oncogene in breast cancer. Only breast cancer cell lines containing high *IKBKE* expression exhibited reduced viability when *IKBKE* levels were reduced by RNAi.

But how exactly does *IKBKE* function to promote cancer? The *IKBKE* gene product (IKK $\epsilon$ ) is a protein that stimulates the activity of IRF-3 (interferon regulatory factor 3) and thus interferon responses to viral infections. Intriguingly, this function was not involved in the oncogenic capacity of *IKBKE*. IKK $\epsilon$  is also known to activate the NF- $\kappa$ B signaling pathway by inducing phosphorylation and nuclear accumulation of cRel, an NF- $\kappa$ B component (Harris et al., 2006). Boehm et al. (2007) determined that IKK $\epsilon$  exerts its oncogenic function through cRel. In

particular, NF- $\kappa$ B-responsive genes were upregulated when IKK $\epsilon$  was deregulated, I $\kappa$ B was destabilized, and nuclear accumulation of cRel was induced. Indeed, inhibition of the NF- $\kappa$ B pathway by overexpression of a degradation-resistant I $\kappa$ B suppressed the transforming activity of *IKBKE*. Activation of the NF- $\kappa$ B pathway has been observed in many breast cancers, but a molecular explanation for this activation has not been clear. The identification by Boehm et al. (2007) of *IKBKE* as an oncogene and activator of the NF- $\kappa$ B pathway in breast cancer explains this observation.

Could IKK $\epsilon$  be a potential target for cancer therapy? Developing and testing inhibitors that block activity of IKK $\epsilon$  should be an interesting future strategy for treating breast cancer. Obviously, the specificity and toxicity of such inhibitors will determine the success of this strategy. It is promising that blocking *IKBKE* expression affected only the viability of cancer cells with deregulated *IKBKE* expression. Interestingly, *IKBKE* has a very close homolog called *TBK1*. Although breast cancer cell lines showed dependence on *IKBKE*, some prostate tumor cell lines were sensitive to inhibition of *TBK1* activity. Whether *TBK1* is an oncogene in prostate cancer, in a manner similar to *IKBKE*'s oncogenic effects in breast cancer, awaits further study. The similarity between these two kinases also suggests that tumor cells treated with *IKBKE* inhibitors could rapidly develop drug resistance through upregulation of *TBK1*. Therefore, the best approach may be to develop an inhibitor capable of blocking the activity of both the IKK $\epsilon$  and *TBK1* kinases.

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# Disrupting the Stem Cell Niche: Good Seeds in Bad Soil

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DOI 10.1016/j.cell.2007.05.053

**Stem cells reside in a microenvironment or niche that is critical for stem cell maintenance and regulation. But what happens when a stem cell niche is disrupted? In this issue of *Cell*, two reports (Walkley et al., 2007a, 2007b) demonstrate in mice that alterations in the niche for hematopoietic stem cells lead to the development of myeloproliferative disease.**

Hematopoietic stem cells (HSCs) are maintained and regulated in microenvironments or niches in the bone marrow. HSCs are known to reside in two different niches, an “osteoblastic” niche and a “vascular” niche. In the osteoblastic niche, HSCs are associated with a subset of osteoblasts (the cells responsible for bone formation) that line the inner surface of the bone cavity (Calvi et al., 2003; Zhang et al., 2003; Arai et al., 2004). In contrast, in the vascular niche, HSCs associate with the surfaces of endothelial cells that line the sinusoids of bone marrow and spleen (Kiel et al., 2005). Recently, it was shown that CXCL12-abundant reticular (CAR) cells are found in association with HSCs in both the osteoblastic and vascular niches (Sugiyama et al., 2006) and may serve as a transit pathway for shuttling HSCs between the two. It has been proposed that these two niches are functionally distinct: the osteoblastic niche is thought to maintain HSC quiescence over the long term, whereas the vascular niche is thought to main-

tain HSCs over a shorter time period, supporting HSC proliferation, favoring myeloid and megakaryocytic lineage differentiation, and mediating HSC circulation (Kopp et al., 2005). Despite the critical role of these two niches in regulating HSCs, evidence for a role of the niche in disease has been limited. In this issue of *Cell*, Orkin, Purton, and their colleagues demonstrate in mice that the microenvironment can play a dominant role in the development of myeloproliferative disease—a disorder characterized by the neoplastic development of myeloid cells (Walkley et al., 2007a, 2007b) (Figure 1).

Given that key cell cycle regulators have been implicated in HSC dysfunction, Orkin and colleagues first examined loss of the retinoblastoma (RB) protein, a cell cycle regulator and tumor suppressor, in the hematopoietic system (Walkley et al., 2007a). Surprisingly, RB was found to be dispensable for self-renewal and multilineage differentiation of HSCs. However, widespread loss of RB in the

hematopoietic system results in extramedullary hematopoiesis (hematopoiesis outside of the bone marrow, for instance, in the spleen). These mice eventually develop myeloproliferative disease. Strikingly, myeloid-specific loss of RB resulted in only mild defects and did not result in myeloproliferative disease or HSC abnormalities, suggesting that the defect resulting from widespread loss of RB is not solely caused by myeloid cells with intrinsic RB deficiency or by myeloid cells derived from RB-deficient HSCs. Furthermore, transplantation of normal hematopoietic cells into an RB-deficient microenvironment failed to recapitulate the effects observed with widespread deletion of RB. Only when myeloid-specific loss of RB was combined with loss of RB in the microenvironment was the full myeloproliferative defect recapitulated. Thus, the myeloproliferative disease observed with widespread loss of RB resulted from an interaction between myeloid cells and the altered microenvironment.