metaplasia (ADM) do, indeed, harbor *KRAS* mutations identical to those observed in adjacent PanIN (Shi et al., 2009). These findings might be consistent with *KRAS* mutations arising in either acinar cells themselves or in areas of ADM, with subsequent rapid progression to PanIN.

Assuming that these findings are indeed relevant to the human disease, what are the ramifications of the current findings? Certainly, they suggest that future chemoprevention strategies might be best targeted at early events in acinar rather than ductal cells; blocking acinar cell activation of *Sox9* now joins Notch pathway inhibition and maintenance of *Mist1* expression as examples of such approaches. In addition, these findings underscore an increasingly recognized disconnect between *Kras* mutations and Kras activity. Along these lines, it will be fascinating to determine the presumably epigenetic determinants underlying the differential responsiveness to oncogenic *Kras* observed in acinar and ductal cell types; manipulating such determinants may convert acinar cells into less capable parents, hopefully eliminating PanIN from the pancreatic family tree.

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Chemokine to the Rescue: Interleukin-8 Mediates Resistance to PI3K-Pathway-Targeted Therapy in Breast Cancer

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Adaptive resistance to PI3K-mTOR inhibitors potentially limits the clinical antitumor activities of these agents. In this issue of *Cancer Cell*, Britschgi and coworkers show that certain tumors acquire resistance to PI3K-mTOR inhibitors through activation of a JAK2-dependent pathway, leading to interleukin-8 secretion.

More than 25 years have passed since the discovery of phosphoinositide 3-kinase (PI3K) as an oncoprotein-associated enzymatic activity. The term "PI3K" in this context designates the Class I subset of phosphoinositide kinases (comprising the α, β, γ, and δ isoforms), which convert phosphatidylinositol-4,5-bisphosphate to the bioactive second messenger phosphatidylinositol-3,4,5-trisphosphate (Vanhaesebroeck et al., 2012). These PI3Ks are activated, directly or indirectly, by a variety of cell surface receptors that include receptor tyrosine kinases (RTKs) and G protein-coupled receptors. Several cardinal alterations elicited by PI3K activation include changes in cell proliferation, survival, migration, and metabolism, and are highly aligned with the "hallmarks of cancer" discussed by Hanahan and Weinberg (2011). Indeed, inappropriate activation of the PI3K pathway has been observed in a remarkably broad array of human cancers. Nested within this pro-oncogenic signaling network are two pivotal protein serine-threonine kinases, AKT (also termed protein kinase B) and mTOR, both of which represent druggable targets, like PI3K itself. This combination of biological relevance and pharmacological tractability rendered the PI3K pathway an irresistible target for cancer drug discovery. The ensuing efforts in

medicinal chemistry delivered a treasure trove of PI3K pathway inhibitors, and a stunning number (25–30) of these compounds are now in various stages of late preclinical or clinical development.

The unprecedented level of pharmaceutical investment in PI3K pathway inhibitors was based on expectations that these agents would exert broad-based inhibitory effects on tumor growth and progression. Unfortunately, single-agent therapy with PI3K pathway inhibitors has, with few exceptions, failed to deliver on the promise of breakthrough antitumor activity in the clinical setting. Adaptive drug resistance has emerged as an important mechanism whereby cancer cells limit the therapeutic activities of these inhibitors (Bagrodia et al., 2012). Recent studies indicate that pharmacological disruption of PI3K signaling engages a multiplicity of compensatory responses that restore signal flux to a level that maintains the oncogenic phenotype. An intriguing report by Britschgi et al. (2012) in this issue of *Cancer Cell* adds to this compendium of resistance mechanisms by demonstrating that certain tumors acquire resistance to PI3K pathway-targeted therapies by elaborating a pro-inflammatory chemokine that not only drives drug resistance, but also leads to a more invasive, metastatic phenotype for the cancer cells.

Britschgi et al. (2012) focused their studies on triple-negative breast cancer (TNBC), a tumor type differentiated from other breast cancer subtypes by the absence of receptors for HER2, progesterone, and estrogen. TNBC is a highly aggressive disease with a poor response to current therapies. Evidence that the PI3K pathway is commonly activated in TNBC has raised hope that PI3K pathway-targeted agents might significantly alter the therapeutic landscape for this disease (Ibrahim et al., 2012). Britschgi et al. (2012) examined the phosphorylation states of specific signaling proteins during acute ($\leq$20 hr) exposure of TNBC-like cell lines to BEZ235, a dually-active PI3K/mTOR inhibitor. An unanticipated finding was that BEZ235 exposure stimulated tyrosine phosphorylation of the JAK2 kinase and its substrate, the STAT5 transcription factor. The JAK kinase family consists of four members, which are typically (but not exclusively) activated by cytokine and chemokine receptors (Seavey and Dobrzanski, 2012). The activated JAKs, in turn, phosphorylate one or more of the seven STAT family proteins, thereby stimulating their nuclear translocation and transactivating functions. BEZ235 treatment triggered selective modification of JAK2 and STAT5, indicating that this drug provoked specific rather generalized activation of JAK-STAT signaling in these TNBC cell lines (Britschgi et al., 2012). Further experiments revealed that BEZ235 stimulated the expression of mRNA transcripts encoding interleukin-8 (IL-8) and the subsequent secretion of this chemokine by the drug-treated cells. The expression of IL-8 receptor CXCR1 was also increased during BEZ235 exposure, establishing an autokine-acting chemokine signaling pathway in the drug-treated TNBC cells.

Exploration of the mechanism underlying BEZ235-induced JAK2-STAT5 activation uncovered a biphasic response. The early (0–8 hr after drug treatment) increase in JAK2-STAT5 activation was linked to upregulation of the IR/IGF-1R signaling pathway. This finding is consistent with previous observations that inhibition of the PI3K-AKT axis of the PI3K signaling network stimulates a FOXO transcription factor-dependent homeostatic response leading to upregulation of receptors that strongly activate PI3K, such as the RTKs IR/IGF1R and MET (Chakrabarty et al., 2012; Muranen et al., 2012). The initial wave of JAK2/STAT5 signaling stimulated the expression and secretion of IL-8, which then acted through the CXCR1 receptor to drive a second wave of JAK2-STAT5 activity. Thus, inhibition of PI3K/mTOR sets in motion a sequence of events that culminate in a self-amplifying autocrine loop involving the IL-8-CXCR1-JAK2-STAT5 signaling cascade. The unanticipated increase in IL-8 signaling provoked by PI3K/mTOR inhibition was at least partially responsible for limiting the inhibitory effects of BEZ235 on AKT activity, cancer cell survival in vitro, and tumor growth in vivo. Moreover, these results may have broader clinical implications, in that IL-8 is recognized as a significant tumor progression factor through its pleiotropic activities related to cancer stem cells, angiogenesis, and metastasis (Waugh and Wilson, 2008). Hence, it is conceivable that treatment of TNBC with PI3K/mTOR inhibitors could to lead to worsened patient outcomes related to enhanced intratumoral IL-8 activity.

Although these findings place an additional obstacle in the path to successful implementation of PI3K pathway-targeted therapies, they also present a therapeutic opportunity, in that the IL8-dependent adaptive response is amenable to pharmacologic intervention (for example with drugs that target JAK2) (Seavey and Dobrzanski, 2012). Indeed, Britschgi et al. (2012) demonstrated that combination therapy with a JAK2 kinase inhibitor increased the antiproliferative and cytocidal activities of BEZ235 in vitro and augmented the antitumor activity of BEZ235 in mouse xenograft studies. Furthermore, the authors observed that circulating tumor cell numbers in mice bearing highly metastatic breast tumors, which were only marginally reduced by the PI3K/mTOR inhibitor, were dramatically suppressed by treatment with a JAK2 inhibitor, both in the absence and presence of BEZ235. The authors further noted that CXCR1 expression was heterogeneous in these tumor cell populations and presented evidence that the most highly CXCR1-positive cells were enriched for both tumor-initiating and metastatic behaviors. The results support a clinically testable combination strategy involving PI3K-mTOR and JAK2 inhibitors in TNBC and in other tumors in which suppression of PI3K signaling provokes a compensatory increase in IL8 production.

This study underscores the robustness of the adaptive network that strives to maintain homeostatic signal flux through the PI3K pathway in cancer cells. Previously described mechanisms of PI3K inhibitor resistance can be characterized as "vertical"; for example, inhibition of PI3K-α elicits a homeostatic response, such as increased expression of IR/IGF1R, which aims to restore PI3K-α activity (Figure 1). The adaptive response uncovered by Britschgi et al. (2012) adds a chemokine-dependent "horizontal" element to the existing array of PI3K inhibitor resistance mechanisms. The more sobering news might be that this adaptive response confers not only drug resistance, but also a more aggressive phenotype on TNBC cells.

This study raises several questions for future investigation. First, given that IL-8

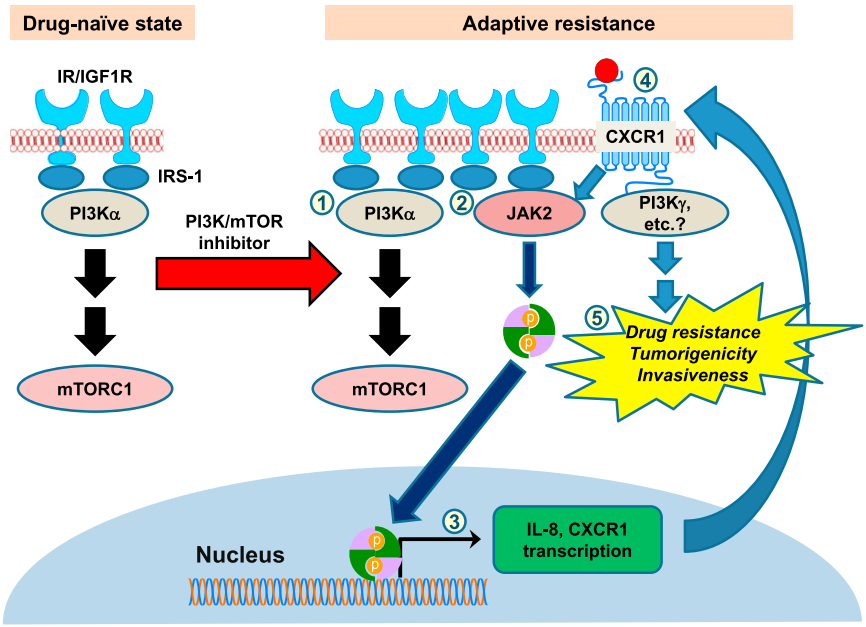

Figure 1. Acquisition of Resistance to PI3K/mTOR Inhibitors through Compensatory Activation of the JAK2-STAT5 Pathway

Triple-negative breast cancer cells drive signaling through the PI3K/mTOR pathway via activation of receptor tyrosine kinases, such as insulin receptors and insulin-like growth factor-1 receptors (IR/IGF-1R). Treatment with a PI3K-mTOR inhibitor triggers a biphasic adaptive resistance response. In the initial phase of this response, the cells upregulate expression of IR/IGF-1R and IRS-1 (1), which stimulates a compensatory increase in PI3K-α activity (vertical resistance), together with IRS-1-dependent activation of the JAK2-STAT5 pathway (2). STAT5 translocates to the nucleus and stimulates transcription of the genes encoding interleukin-8 (IL-8) and the IL-8 receptor, CXCR1 (3). The secretion of IL-8 establishes a self-amplifying, autocrine and/or paracrine chemokine loop that might activate a different PI3K isoform, PI3K-γ (4), and propagates JAK2-STAT5 activation, leading to horizontal drug resistance and increased tumorigenicity and invasiveness in TNBC cell populations (5).

signals through a GPCR, it would be interesting to learn whether the IL-8-dependent adaptive response involves a switch from PI3K-α to the GPCR-associated PI3K-γ isoform (Figure 1). Second, PI3K/mTOR inhibitors stimulate autophagy, which was recently shown to support the secretion of IL-8 and other pro-inflammatory cytokines through an unconventional secretory pathway (Deretic et al., 2012). If IL-8 release from drug-treated TNBC cells is attributable to unconventional secretion, then this mechanism of adaptive resistance might be susceptible to combination therapy with an autophagy inhibitor. Finally, and most importantly, we need to understand more fully the actual contributions of IL-8 to PI3K/mTOR inhibitor resistance in human TNBC patients, as well the roles of drug-induced IL-8 release in adaptive drug resistance in other human cancers.

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