Breast Cancer
R E S E A R C H

VIEWPOINT

Closing escape routes: inhibition of IL-8 signaling enhances the anti-tumor efficacy of PI3K inhibitors

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Abstract

The phosphoinositide 3-kinase (PI3K) pathway serves as a relay where signals that emanate from the cell membrane are received and converted into intracellular signals that promote proliferation and survival. Inhibitors of PI3K hold promise for the treatment of breast cancer because activation of this pathway is highly prevalent. However, as is increasingly observed with inhibitors of cell signaling, there appear to be mechanisms of primary and secondary resistance. Britschgi and colleagues report that compensatory activation of the IL-8 signaling axis is a mechanism of primary resistance to PI3K inhibitors in some triple-negative breast cancers. In a set of experiments that carefully emulate the clinical scenario in a mouse model, they show that simultaneous inhibition of Janus kinase 2 enhances the efficacy of PI3K/mammalian target of rapamycin inhibition. Their paper lends further support to the concept that successful design of treatments with signal transduction inhibitors must anticipate potential escape routes – and include agents to simultaneously block them.
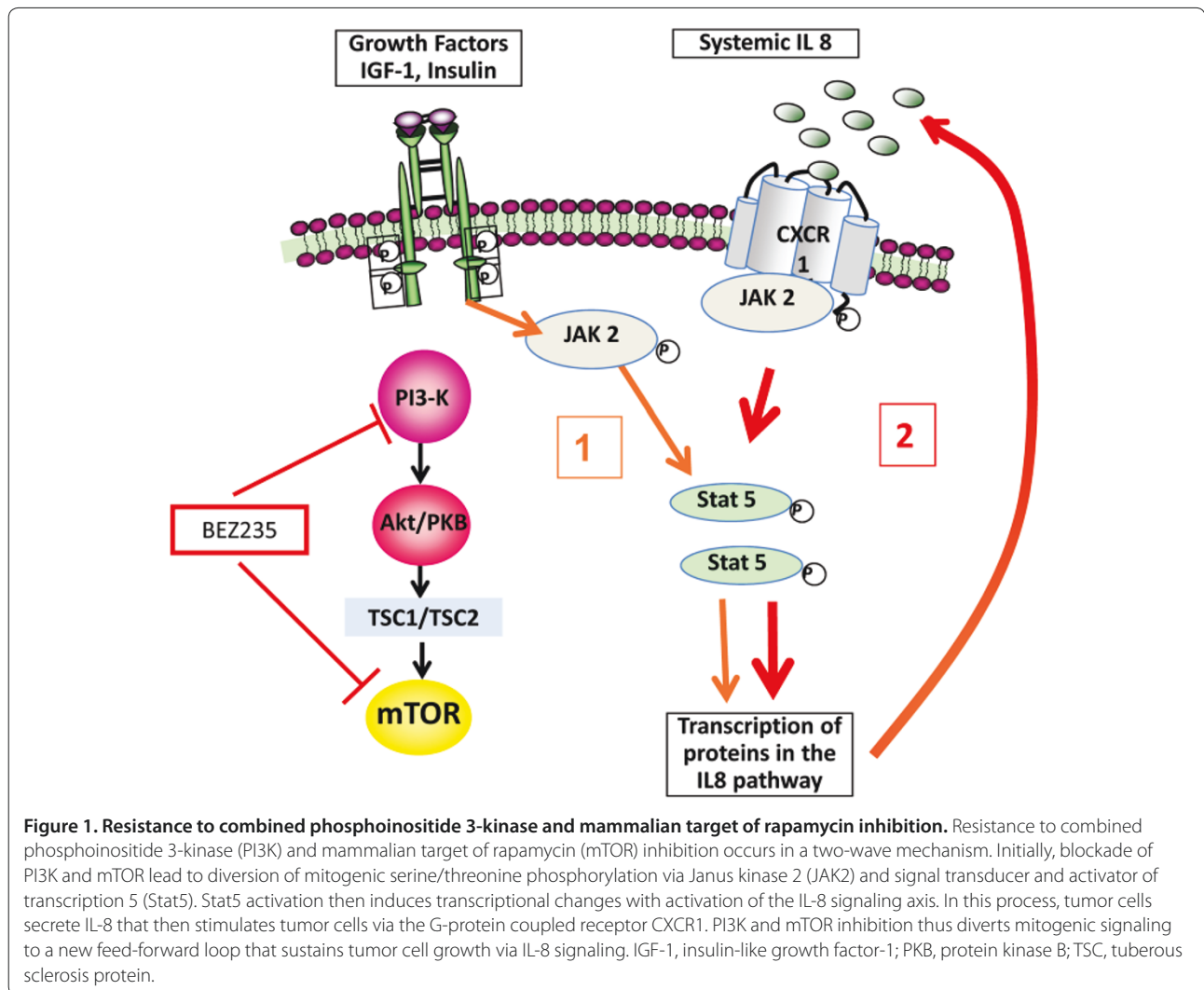
Metastatic triple-negative breast cancer (TNBC) remains an unsolved clinical problem. Patients are typically treated with cytotoxic chemotherapy that severely impedes their quality of life, response rates decline with each subsequent treatment regimen, and even when remission is achieved it is temporary and tumor progression occurs within a few months. The promise of targeted treatments is that, because of their specificity for the tumor cell's signaling machinery, they could potentially suppress tumor growth for a prolonged period and at lesser cost to quality of life. Two key challenges in the design of such studies are finding the right target and anticipating and counteracting resistance mechanisms.

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There is a good rationale to target phosphoinositide 3-kinase (PI3K) in breast cancer, including TNBC: 30 to 40% of estrogen receptor-positive breast cancer, 20 to 30% of Her2-amplified breast cancer, and 7 to 20% of TNBC have activating mutations of PIK3CA (encoding the p110α subunit of PI3K) [1-4]. While the frequency of activating mutations in PIK3CA is relatively low in TNBC, an increase in epidermal growth factor receptor expression [5,6] and inactivation of the inhibitory phosphatases PTEN and INPP4B [7,8] are frequent, and thus activation of the PI3K pathway is also highly prevalent in TNBC.

These findings have led to a number of preclinical and now ongoing clinical studies examining the efficacy of PI3K inhibitor monotherapy and, anticipating resistance to PI3K/mammalian target of rapamycin (mTOR) monotherapy, of combination therapies that include Parp inhibitors [9,10] or MEK inhibitors [11]. In a set of elegant experiments that tries to recapitulate clinical scenarios closely *in vitro* and in a mouse model, Britschgi and colleagues examined the biological basis for resistance to PI3K/mTOR inhibition in TNBC [12].

Britschgi and colleagues show that inhibition of PI3K not only rewires intracellular signaling but also leads cancer cells to recruit alternate extracellular signaling mechanisms to circumvent PI3K (Figure 1). This inhibition occurs in a two-step process: within hours of exposure to the dual PI3K/mTOR inhibitor NVP-BEZ 235, TNBC cells responded with upregulation of insulin-receptor signaling and with its downstream effector IRS1 directly activating Janus kinase 2 (JAK2) and its substrate, the transcription factor signal transducer and activator of transcription 5 (STAT5). Presumably through changing the transcriptional profile of the cancer cells, STAT5 then causes a more sustained upregulation of the IL-8 signaling axis, including secretion of IL-8 and upregulation of its receptor CXCR1 that then takes over to maintain JAK2/STAT5 signaling (Figure 1). The net effect is that cancer cells which typically rely on receptor tyrosine kinases/PI3K signaling now shift to G-protein coupled receptors, in this case IL-8/CXCR1, to activate JAK2/STAT5 and to keep their mitotic machinery going. The biological significance of this stepwise transition

Figure 1. Resistance to combined phosphoinositide 3-kinase and mammalian target of rapamycin inhibition. Resistance to combined phosphoinositide 3-kinase (PI3K) and mammalian target of rapamycin (mTOR) inhibition occurs in a two-wave mechanism. Initially, blockade of PI3K and mTOR lead to diversion of mitogenic serine/threonine phosphorylation via Janus kinase 2 (JAK2) and signal transducer and activator of transcription 5 (Stat5). Stat5 activation then induces transcriptional changes with activation of the IL-8 signaling axis. In this process, tumor cells secrete IL-8 that then stimulates tumor cells via the G-protein coupled receptor CXCR1. PI3K and mTOR inhibition thus diverts mitogenic signaling to a new feed-forward loop that sustains tumor cell growth via IL-8 signaling. IGF-1, insulin-like growth factor-1; PKB, protein kinase B; TSC, tuberous sclerosis protein.

from receptor tyrosine kinases/PI3K to G-protein coupled receptor/JAK2 mitogenic signaling is confirmed by the findings that concomitant blockade of PI3K/mTOR and IL-8 signaling could effectively decrease tumor growth and metastasis and improve disease-free and overall survival in mice.

Britschgi and colleagues' findings illustrate the plasticity of the signaling mechanisms that drive cancer cells: PI3K/mTOR inhibition is acutely compensated for by the recruitment of IRS1/JAK2/STAT5 phosphorylation and eventually by a change of the transcriptional program in a way that leads to independence from PI3K signaling. They show a pattern of resistance development where cancer cells immediately adapt with a change in phosphorylation routes, followed by transcriptional reprogramming. For the practical purposes of cancer treatment the question really is just how many escape routes there are for cancer cells to evade monotherapy with a targeted agent, and specifically PI3K inhibition. Notably,

while disease-free and overall survival was increased, none of the tumors in Britschgi and colleagues' model were cured by the PI3K/JAK2 inhibitor combination. This failure to cure disease means that tumor cells found a third way around PI3K inhibition, and it is intriguing to speculate that this third wave of resistance may be due to the evolution of genetically resistant clones that, for example, harbor a myc amplification [13].

Will efforts to treat breast cancer with combinations of signal transduction inhibitors be akin to the boy who is trying to hold the flood with his fingers in the dyke? The observed order of events with immediate adaptation of signaling pathways over transcriptional reprogramming and eventually presumed outgrowth of genetically resistant clones suggests that there may be a finite number of escape pathways, and inhibitors to target them may already be available. In addition, this novel approach might target specific tumor cell populations that are not typically eradicated by chemotherapy. The fact that

combined JAK2 plus PI3K/mTOR inhibition greatly de-creased circulating tumor cells, and specifically meta-stasis formation, suggests that this approach could poten-tially enhance the efficacy of cytotoxic chemotherapy.

A number of findings in Britschgi and colleagues' work raise immediate clinically important questions. Only some TNBC cell lines responded to PI3K inhibition with an increase in JAK2/phosphorylated-STAT5 signaling – is there a common genetic denominator that dictates this response? Could measurement of blood IL-8 levels identify patients whose tumors might be *de novo* resistant to PI3K inhibition? Would concomitant blockade of IL-8 receptors, important for neutrophil function, increase susceptibility to bacterial infections?

Rashes and non-infectious pneumonitis are known class effects that occur in patients treated with mTOR inhibitors [14], and may also occur with PI3K inhibitors. Given the significance of IL-8 levels as a biomarker for pneumonitis, it is interesting to speculate that compen-satory IL-8 production might actually be the mechanism for this thus far unexplained toxicity, and, if so, conco-mitant blockade of IL-8 signaling and PI3K might actually reduce pulmonary toxicity. Lastly, there is no reason to assume that upregulation of IL-8 signaling upon PI3K inhibition is specific to breast cancer, and upregulation of the IL-8 signaling axis should be considered a potential resistance mechanism in all cancers where PI3K inhi-bition is currently being studied.

Abbreviations

JAK2, Janus kinase 2; IL, interleukin; mTOR, mammalian target of rapamycin; PI3K, phosphoinositide 3-kinase; STAT5, signal transducer and activator of transcription 5; TNBC, triple-negative breast cancer.

Competing interests

The authors declare that they have no competing interests.

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