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AUTHOR'S VIEW

Epigenetic inhibition of adaptive bypass responses to lapatinib by targeting BET Bromodomains

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ABSTRACT
The characterization of kinases as oncogenic drivers has led to more than 30 FDA-approved targeted kinase inhibitors for cancer treatment. Unfortunately, these therapeutics fail to have clinical durability because of adaptive responses from the kinome and transcriptome that bypass inhibition of the targeted pathway. In our recent work, we describe a method to prevent these adaptive responses at an epigenetic level, generating a durable response to kinase inhibition.

Abbreviations: AKT, v-akt murine thymoma viral oncogene homolog; BET, bromodomain and extra-terminal; BRD4, bromodomain containing 4; ChIP, Chromatin immunoprecipitation; DDR1, discoidin domain receptor tyrosine kinase 1; ERK, extracellular signal-regulated kinase; FAK, focal adhesion kinase; FGFR2, fibroblast growth factor receptor 2; HER, human epidermal growth factor receptor; IGF1R, insulin-like growth factor 1 receptor; INSR, insulin receptor; MAPK, mitogen-activated protein kinase; MEK, mitogen/extracellular signal-regulated kinase kinase; MET, hepatocyte growth factor receptor; RNAseq, Illumina deep mRNA sequencing; RTK, receptor tyrosine kinase; SFK, SRC family kinase; SRC, v-Src avian sarcoma (Schmidt-Ruppin A-2) viral oncogene homolog; TNBC, triple-negative breast cancer

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Many cancer patients initially benefit from clinical treatment with small molecule kinase inhibitors, but tumors often develop resistance to these agents. Adaptive responses from the kinome and transcriptome serve to reactivate the targeted pathway or initiate bypass tracks that ultimately limit the durability of targeted therapies. These adaptive responses are a fundamental result of targeting oncogenic nodes within tumors and are a significant clinical problem in the treatment of cancer patients.[1] Such responses are based in part on the disruption of feedforward and feedback regulatory loops that control transcription and the activity of many signaling networks.[2-5] In triple-negative breast cancer (TNBC) for example, inhibition of MEK/ERK signaling with the small molecule MEK inhibitor selumetinib (AZD6244) leads to degradation of c-Myc and relief of transcriptional repression of several receptor tyrosine kinases (RTKs).[4] Upregulation and activation of these RTKs reactivates the MAPK pathway leading to drug resistance. Targeting this compensatory response with the multitargeted kinase inhibitor sorafenib in combination with selumetinib inhibited the activity of adaptive kinases, leading to cell death and apoptosis in a TNBC mouse model. Targeting the activity of responsive kinases has also shown similar synthetic lethality in other cancer types.[6-8]

In our recent work published in *Cell Reports,* we used a chemical proteomics method to define global adaptive changes in kinome activity in a series of HER2-amplified cell lines treated with the HER2 inhibitor lapatinib.[9] We identified compensatory activation of HER3 and SRC family kinases (SFKs), consistent with previous reports,[2,5,10] but also observed a dramatic response from the majority of the kinome. We found a series of tyrosine kinases that were significantly activated following inhibition of HER2, including FAK, DDR1, FGFR2, IGF1R, INSR, and multiple ephrin receptors. RNAseq demonstrated that several of the RTKs were transcriptionally upregulated, and surprisingly nearly 20% of the transcriptome was dysregulated, indicating a major reorganization of kinase signaling pathways and transcriptional regulatory networks. Additionally, we found significant heterogeneity in the adaptive responses toward lapatinib across cell lines. When targeting the compensatory kinases with a series of tyrosine kinase inhibitors in 4-week colony formation assays we found variable sensitivity to combination therapies across cell lines. Proteomic profiling of cells that were resistant to lapatinib again identified multiple kinases that were overactivated including HER3, IGF1R, MET, DDR1, multiple FGFRs, FAK, and SFKs. siRNA-mediated knockdown of these kinases indicated incremental contributions to the growth of lapatinib-resistant cells, with no dominant driver of proliferation. Most of these kinases were activated or upregulated within 48 h of lapatinib treatment in parental cells, indicating that multiple mechanisms of resistance emerge rapidly upon HER2 inhibition. Targeting AKT, a downstream nodal kinase, in combination with lapatinib did enhance growth inhibition, but actually amplified the RTK response beyond that of lapatinib alone while also inducing strong activation of MEK/ERK signaling. Concordant with these findings, colonies that were resistant to the combination of lapatinib plus AKT inhibitor developed.

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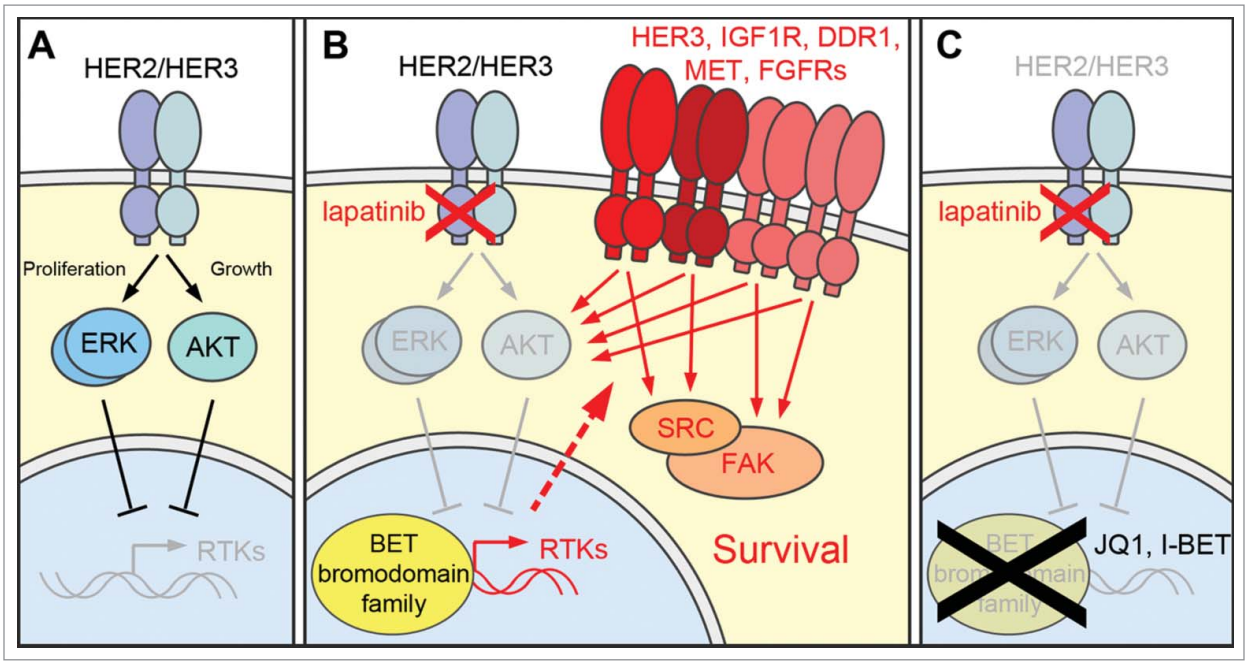

Figure 1. BET bromodomain inhibition prevents adaptive responses to lapatinib. (A) The HER2/HER3 oncogenic dimer drives the ERK and AKT signaling networks responsible for tumor cell growth and proliferation. (B) Inhibition of HER2 activity with the small molecule tyrosine kinase inhibitor lapatinib relieves transcriptional repression of receptor tyrosine kinases (RTKs). Multiple RTKs are upregulated and reactivate ERK/AKT and promote bypass tracks via SRC/FAK signaling, leading to cell survival. Heterogeneity in the response and functional redundancy among adaptive RTKs limits the ability to effectively target the compensatory signaling. (C) Targeting the machinery responsible for RTK induction, the BET bromodomain-containing family of chromatin readers, prevents the adaptive response at an epigenetic level. Combining BET bromodomain inhibitors (JQ1, I-BET151, I-BET762) with lapatinib makes kinase inhibition durable by blocking the transcription of kinases involved in the adaptive response.

From this comprehensive analysis we were confronted with a dilemma, in which combinations of 2, or even 3, kinase inhibitors would be insufficient to suppress the resiliency of the kinome and potentiate growth inhibition. We reasoned instead that the adaptive response itself must be blocked for the effect of lapatinib to be durable. We decided to target the transcriptional response at an epigenetic level to prevent the initial upregulation of RTKs. We found that BET bromodomain inhibitors including JQ1, I-BET151, and I-BET762 inhibited the lapatinib-mediated induction of *HER3*, *DDR1*, *IGF1R*, *FGFR2*, and *MET* across the panel of HER2-positive cell lines, preventing FAK and SFK phosphorylation and AKT reactivation (Fig. 1). Long-term growth assays indicated all of the cell lines were either growth arrested or underwent cell death with the combination of lapatinib and BET bromodomain inhibitor. RNAseq revealed that JQ1 downregulated approximately 8% of all expressed genes when used alone but suppressed 27% of lapatinib-induced genes in combination treatment, indicating that BET bromodomain inhibitors preferentially inhibit induced gene expression. ChIP-PCR demonstrated that JQ1 maximally dissociated the BET bromodomain protein BRD4 and RNA polymerase II from the promoters of responsive RTKs when combined with lapatinib, indicating molecular synergism between the agents at an epigenetic level. BET bromodomain inhibitors even suppressed RTK expression in lapatinib-resistant cells, effectively reversing the adaptive response. BET bromodomain inhibitors arrested growth and were superior to combinations of other kinase inhibitors in growth assays with lapatinib-resistant cells, but removal of lapatinib from the media while maintaining BET inhibitors allowed the cells to begin to grow again. This indicated that BET inhibitors actually resensitized resistant cells to lapatinib by suppressing the drivers of bypass pathways.

We believe these findings will serve as a paradigm shift in how combination therapies are approached in the clinic, as these adaptive bypass mechanisms are fundamental to every cancer type. We described a select set of RTKs that were induced across several HER2-positive cell lines and all had the potential to drive resistance. These RTKs, which are targeted by BET bromodomain inhibitors, are crucial to the growth and resistance mechanisms of many other cancers, suggesting the addition of BET inhibitors to other targeted therapies might improve their efficacy. Multiple BET bromodomain inhibitors are moving forward in clinical trials, offering the potential to test this combination in patients. Our study serves as a proof-of-concept, and an important next step in the successful design and implementation of such combination therapies will be a thorough analysis of the epigenetic changes driven by targeted kinase inhibition. What is the nature of the DNA and histone modifications that occur following the disruption of kinase signaling networks, and how do the kinome and the epigenome interact? A more thorough cataloguing of kinases that respond to lapatinib, as well as other targeted therapies, will be crucial to directing this research, and specific kinase inhibitors will likely dictate the epigenetic enzymes to be blocked.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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