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Inhibition of lapatinib-induced kinome reprogramming in ERBB2-positive breast cancer by targeting BET family bromodomains

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Graphical Abstract
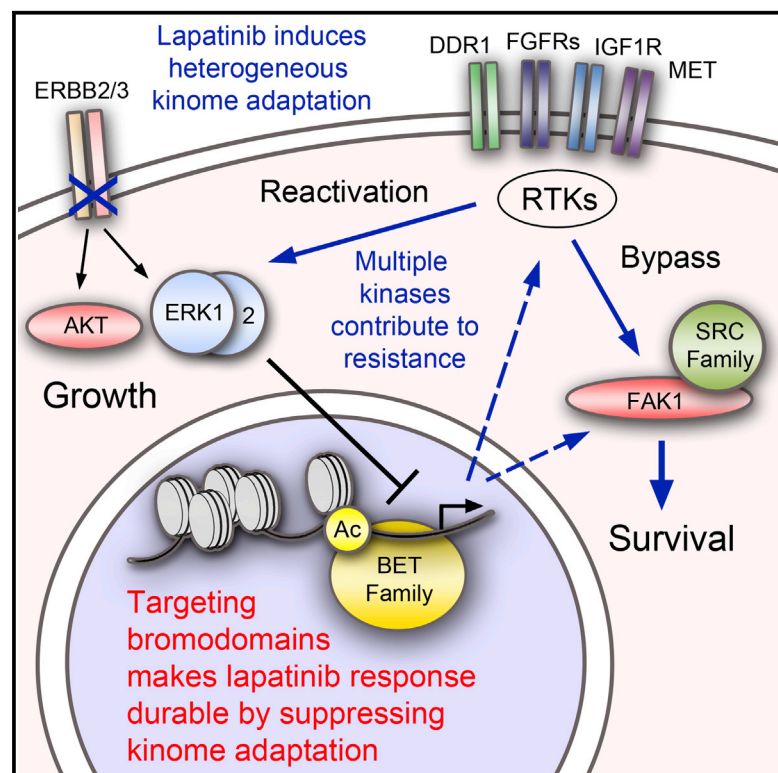
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In Brief

Signaling rewiring within cancer cells is a common mechanism of drug resistance. Stuhlmiller et al. describe a method to prevent this rewiring by inhibiting BET bromodomains, making the initial drug effect durable in inhibiting tumor cell growth

Highlights

- Lapatinib induces heterogeneous RTK-based kinome adaptation in ERBB2^{+} cells

- Multiple unrelated kinases contribute to cell growth in the presence of lapatinib

- BET bromodomain inhibition suppresses expression of lapatinib-induced kinases

- Targeting kinome adaptation makes kinase inhibition durable

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Inhibition of Lapatinib-Induced Kinome Reprogramming in ERBB2-Positive Breast Cancer by Targeting BET Family Bromodomains

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SUMMARY

Therapeutics that target ERBB2, such as lapatinib, often provide initial clinical benefit, but resistance frequently develops. Adaptive responses leading to lapatinib resistance involve reprogramming of the kinome through reactivation of ERBB2/ERBB3 signaling and transcriptional upregulation and activation of multiple tyrosine kinases. The heterogeneity of induced kinases prevents their targeting by a single kinase inhibitor, underscoring the challenge of predicting effective kinase inhibitor combination therapies. We hypothesized that, to make the tumor response to single kinase inhibitors durable, the adaptive kinome response itself must be inhibited. Genetic and chemical inhibition of BET bromodomain chromatin readers suppresses transcription of many lapatinib-induced kinases involved in resistance, including ERBB3, IGF1R, DDR1, MET, and FGFRs, preventing downstream SRC/FAK signaling and AKT reactivation. Combining inhibitors of kinases and chromatin readers prevents kinome adaptation by blocking transcription, generating a durable response to lapatinib, and overcoming the dilemma of heterogeneity in the adaptive response.

INTRODUCTION

The human epidermal growth factor receptor 2 (HER2/ERBB2) is a member of the EGFR/ERBB family of receptor tyrosine kinases (RTKs). The *ERBB2* oncogene is amplified or overexpressed in roughly 25% of breast cancers and serves as the primary driver of tumor cell growth in the majority of these cancers. Clinical trials indicate that ERBB2 "addiction" is fundamental to the behavior of these tumors, and targeting ERBB2 has proved to be an effective treatment in a subset of ERBB2^{+} breast cancer patients. Approved ERBB2-targeting therapies include the monoclonal antibodies trastuzumab, pertuzumab, and trastuzumab-DM1, an antibody drug conjugate, in addition to the ATP-competitive EGFR/ERBB2 inhibitor lapatinib. However, even with initial dramatic clinical responses to these therapies as single agents or in combination, patients frequently relapse as resistance develops. The preferred dimerization partner of ERBB2 is ERBB3/HER3, and a major mechanism of lapatinib resistance is due to transcriptional and post-translational upregulation of ERBB3 (Amin et al., 2010; Garrett et al., 2011). Multiple other kinases contribute to the resistant phenotype as well, including IGF1R, MET, FGFR2, FAK, and SRC family kinases (Azuma et al., 2011; Huang et al., 2011; Rexer and Arteaga, 2012).

Characteristically, tumors have a remarkable resiliency toward kinase-directed therapeutics, capable of rewiring their signaling networks to evade effects of the drug and develop resistance. Targeting specific signaling nodes crucial for tumor growth, such as PI3K, AKT, mTOR, BRAF, and MEK, elicits adaptive kinome responses that upregulate alternative kinase signaling networks or reactivate the targeted pathway to overcome inhibitor treatment (Chandarlapaty et al., 2011; Duncan et al., 2012; Rodrik-Outmezguine et al., 2011; Serra et al., 2011; Sun et al., 2014). This "adaptive kinome reprogramming" is mechanistically based on the disruption of feedback and feedforward regulatory loops that serve to bypass the inhibition and rapidly generate resistance to targeted therapies. Adaptive bypass responses in tumor cells are a major reason that kinase inhibitors often do not have durable responses in the treatment of cancer patients.

To understand these bypass mechanisms toward ERBB2 inhibition, we investigated lapatinib-induced kinome adaptation in a panel of ERBB2^{+} cell lines using a chemical proteomics method.

We find the adaptive kinome response to lapatinib involves the activation of multiple RTKs, SRC family kinases, FAK, and members of other intracellular networks downstream of RTKs. We additionally identify significant heterogeneity in this response among different ERBB2$^+$ cell lines. Multiple kinases contribute to escape from lapatinib-mediated growth inhibition, consistent with a shift in dependency to alternative signaling nodes in addition to ERBB2. This prevents their targeting by a single kinase inhibitor, underscoring the difficulty of choosing the most effective kinase inhibitor combinations to treat ERBB2$^+$ tumors. These results suggest that chasing combination therapies with multiple kinase inhibitors has a poor likelihood of success. We approached this problem with the hypothesis that lapatinib would be more durable in inhibiting ERBB2$^+$ cell growth if we could block the adaptive reprogramming response itself. We target chromatin readers involved in transcriptional upregulation of RTKs that drive the adaptive signaling networks responsible for lapatinib resistance. By inhibiting the onset of the adaptive response, we achieve durable growth inhibition greater than that observed by targeting several different kinases with inhibitors. Our studies demonstrate that inhibiting the adaptive kinome response provides a method to address the heterogeneity in kinome adaptation and a mechanism to prevent resistance to kinase inhibitors.

RESULTS

Lapatinib Induces Dynamic Adaptive Kinome Responses in ERBB2$^+$ Breast Cancer Cells

We used multiplexed inhibitor beads coupled with mass spectrometry (MIB/MS) to quantitatively measure dynamic changes in kinase activity on a proteomic scale (Figure 1A) (Duncan et al., 2012). SKBR-3 and BT474 luminal ERBB2$^+$ breast cancer cells were treated with lapatinib for 4, 24, and 48 hr (Figures 1B, S1A, and S1B). The kinome of SKBR-3 cells is remarkably responsive to lapatinib with many kinases displaying enhanced MIB binding, indicative of increased kinase activity relative to untreated cells. Lapatinib induces growth inhibition, and there is a concomitant time-dependent loss of MIB binding of cell-cycle-regulating kinases, correlating with inhibition of their kinase activity. Loss of ERBB2 and EGFR MIB binding was observed in both SKBR-3 and BT474 cells at 4 hr, but in SKBR-3 cells, ERBB2 binding had returned to untreated levels after 48 hr, indicating reactivation of ERBB2 (Figures 1B and 1C). In BT474 cells, ERBB2 remains inhibited at 48 hr. ERBB3 binding to MIBs increases within 24 hr, consistent with ERBB3 upregulation in response to lapatinib (Amin et al., 2010). The time course illustrates the dynamic behavior of the kinome with kinases having temporal differences in regulation of their activity (Figures 1D, 1E, and S1A). EGFR displays rapid and sustained loss of MIB binding, while most inhibited kinases demonstrate a progressive loss of MIB binding over 48 hr of lapatinib treatment. Some kinases display similar reactivation dynamics to ERBB2 (IGF1R, PTK6, ADCK1), suggesting they operate in a common regulatory network. Others such as ERBB3, AKT1, DDR1, and ARAF are initially inhibited but reactivate with greater MIB binding than their baseline control activity. Kinases such as FGFR2 respond with increased activity within 4 hr, while other tyrosine kinases
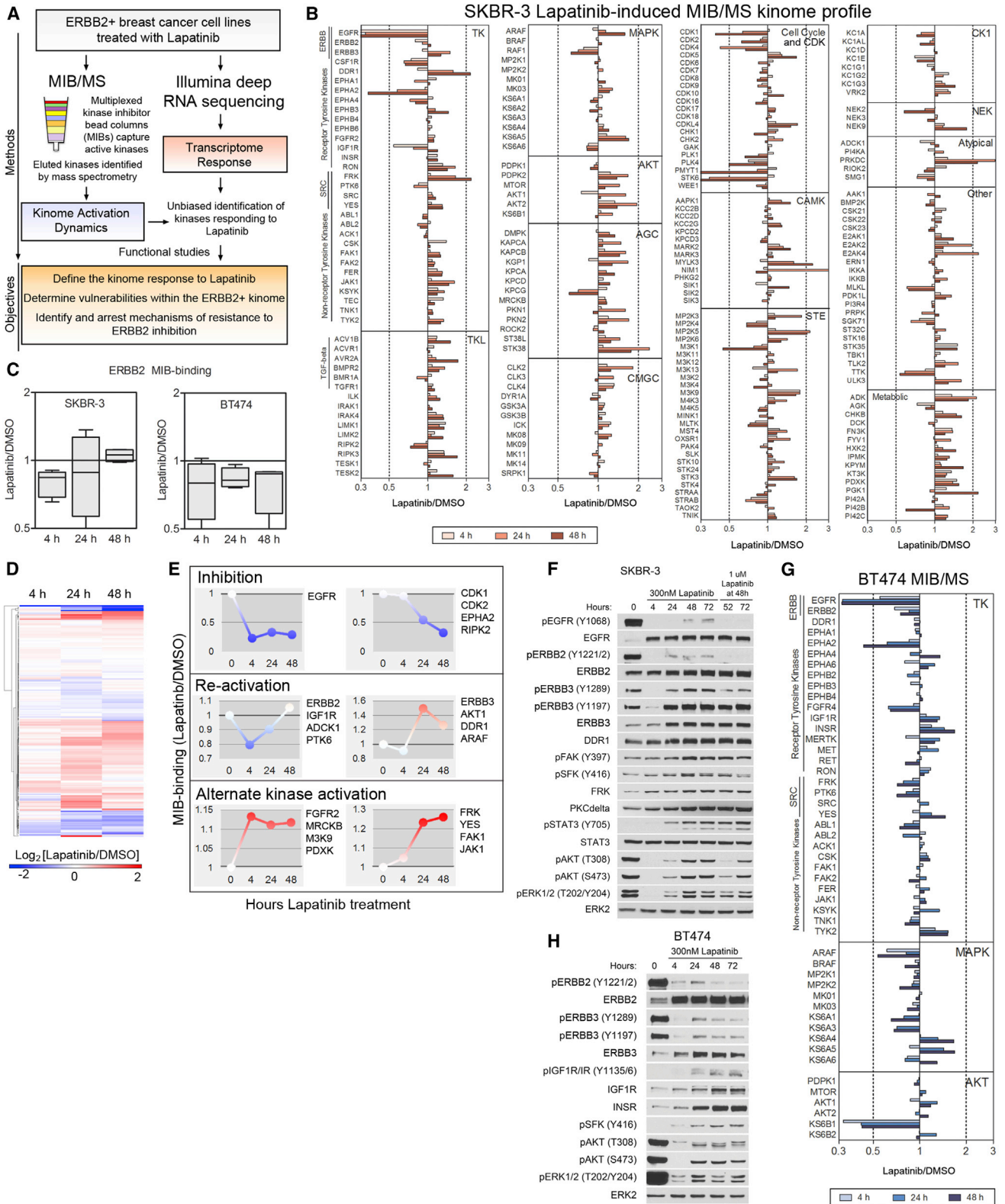
(TKs) such as FRK, YES, FAK1, and JAK1 become progressively more activated, suggesting they are regulated downstream of the kinases driving the initial adaptive kinome response. Western blots confirmed MIB/MS results, with inhibition of ERBB family phosphorylation and downstream signaling (AKT, ERK1/2) at 4 hr and reactivation by 48 hr (Figure 1F). Total protein levels of EGFR, ERBB2, ERBB3, DDR1, FRK, and PKCδ increase over time, along with activation loop phosphorylation of FAK and SRC family kinases (SFKs). STAT3 activating phosphorylation was induced by lapatinib, downstream of JAK signaling and independent of SFK signaling (Figure S1C). Addition of a higher dose of lapatinib (1 μM) after 48 hr inhibited partial reactivation of EGFR, ERBB2, and ERBB3 phosphorylation, but effects on downstream FAK/SFK/AKT/ERK signaling were limited.

BT474 cells generated a less-robust adaptive response with more kinases inhibited than activated (Figures 1G and S1B). BT474 cells displayed a progressive loss of MEK/ERK MIB binding, but a rebound in AKT signaling similar to SKBR-3 cells, with initial inhibition of AKT1 and overall increase in AKT2 MIB binding (Figures 1B and 1G). Interestingly, MEK/ERK MIB binding in SKBR-3 cells was seemingly unchanged by short-term treatment with lapatinib in the 4-hr MIB/MS profile, suggesting additional inputs regulate their activity. Western blots of BT474 cells treated with lapatinib demonstrated little reactivation of ERBB2 and ERBB3, with progressively increasing IGF1R and INSR phosphorylation and total levels, SFK phosphorylation, and a partial return of AKT and ERK1/2 activity (Figure 1H).

MIB/MS Defines Heterogeneity in the Adaptive Kinome Response to Lapatinib

Across four independent MIB/MS runs for SKBR-3 cells, we defined a signature of kinases with highly statistically significant changes in MIB binding after 48-hr lapatinib treatment (Figure 2A). Kinases with enhanced MIB binding (increased activity) include the RTKs DDR1, EPHB3, and FGFR2, non-receptor TKs JAK1, FAK1, and SFKs FRK and YES, and multiple kinases involved in cytoskeletal regulation (MYLK3, NEK9, MARK2, MRCKB, LIMK2). The CMGC kinases CDK5, -10, and -17 and AGC kinases KPCD (PKCδ) and KS6A5 (RSK5) are also activated. PRKDC (DNA damage sensor), STK3 (HIPPO pathway/pro-apoptotic signaling), and AAPK1 (AMP-activated) are activated by multiple growth-inhibiting treatments and likely represent a stress-induced kinase response. Kinases with loss of MIB binding within the signature include multiple cell-cycle-regulating kinases, RTKs EGFR and EPHA2, and serine/threonine kinases KC1A, RIPK2, M3K2, and KS6A1 (RSK1). For BT474 cells, the 48-hr MIB/MS signature defines a very different lapatinib response, with activation of INSR, PRPK (TP53-regulating kinase), ULK3 (autophagy, Hedgehog pathway), and KS6A4 (RSK4). Opposite from SKBR-3 cells, DDR1, FRK, NEK9, and cytoskeleton-regulating kinases (MARK3 and LIMK1) were inhibited (Figure 2A). Of activated kinases, only YES, KS6A5, PRKDC, and STK3 are common between SKBR-3 and BT474 cells.

MIB/MS was performed for three additional ERBB2-amplified cell lines after 48-hr lapatinib treatment: luminal HCC1419 cells are highly sensitive to lapatinib-induced growth arrest, while

A

Methods / Objectives flowchart:

ERBB2+ breast cancer cell lines treated with Lapatinib

MIB/MS → Multiplexed kinase inhibitor bead columns (MIBs) capture active kinases → Eluted kinases identified by mass spectrometry

Illumina deep RNA sequencing → Transcriptome Response

Kinome Activation Dynamics

Unbiased identification of kinases responding to Lapatinib

Functional studies

Objectives:
Define the kinome response to Lapatinib
Determine vulnerabilities within the ERBB2+ kinome
Identify and arrest mechanisms of resistance to ERBB2 inhibition

B SKBR-3 Lapatinib-induced MIB/MS kinome profile

C ERBB2 MIB-binding

D

E

Inhibition: EGFR; CDK1, CDK2, EPHA2, RIPK2

Re-activation: ERBB2, IGF1R, ADCK1, PTK6; ERBB3, AKT1, DDR1, ARAF

Alternate kinase activation: FGFR2, MRCKB, M3K9, PDXK; FRK, YES, FAK1, JAK1

F SKBR-3

G BT474 MIB/MS

H BT474

(legend on next page)

basal-like HCC1954 and luminal MDA361 cells are more resistant. HCC1954 and MDA361 harbor activating *PIK3CA* mutations (H1047R and E545K, respectively) and display resistance to trastuzumab. The kinome of HCC1954 cells is very responsive with activation of multiple RTKs, PKC isoforms, and CAMKs. In contrast, the kinome of MDA361 cells was mostly suppressed by lapatinib, with INSR and IGF1R the only RTKs with statistically significant increases in MIB binding (Figure 2A). HCC1419 cells are intermediate in their adaptive response, with four TKs significantly activated: DDR1, INSR, FAK1, and FRK. Across cell lines, multiple induced kinases are known to modulate or act downstream of ERBB signaling, including SFKs, FAK1, JAK1, CSK, CDK5, and PKCδ, emphasizing an addiction to ERBB-driven signaling networks (Allen-Petersen et al., 2014; Li et al., 2003). It was unexpected that the lapatinib adaptive response would demonstrate such heterogeneity across multiple kinase subfamilies in five ERBB2+ cell lines (Figure 2B, TKs; Figure S2A, other subfamilies). The responsiveness of the kinome does not seem to correlate with EGFR/ERBB2 expression or activation level or the dependency on different ERBB family members as measured by RNAi analysis (Figures S2B and S2C).

The RTK family displays significant variability in activity and response across the five cell lines, but IGF1R/INSR is commonly activated and EPHA2 is commonly inhibited in all lines (Figure 2B). Among non-receptor TKs, multiple cell lines activate FRK, FAK1, and TYK2. SKBR-3 and HCC1954 share a robust activation of most TKs captured by MIBs. 3×3 self-organizing map (SOM) clustering identifies common kinase behavior between several lines, including induction of PRKDC, STK38, NEK9, FRK, STK3, and DDR1 (Figures S3A and S3B). A cluster preferentially induced in HCC1954 includes stress response kinases (MK09, MK11, MK14, STK24, CSK22 (CK2α), KCC2G (CAMK2G), KPCD2, and TKs ACK1 and PTK6 (Figure S3C). Commonly inhibited kinases among the five cell lines include cell-cycle-regulating kinases as well as KS6B1 (p70 S6 kinase) (Figure S3D). To understand the variation in kinome response between cell lines, we utilized principal component analysis (PCA) (Figure 2C). Principal component 1 (PC1) accounts for the majority of the variation and separates kinases that are commonly suppressed (STK6, PMYT1, CDK1, EPHA2, CDK4) from those that are primarily induced (PRKDC, INSR, DDR1) among cell lines. Kinases driving variation between cell lines in PC2 and PC3 agree with differences observed in 48-hr MIB/MS signatures (Figure 2A)

and SOM analysis (Figures S3A–S3D) and provide a statistical measure of the heterogeneity in the kinome response. These kinases include DDR1, FRK, KPCD, KT3K, CDK5, PTK6, PRPK, and KS6A4.

mRNA sequencing (RNA-seq) indicated 18%–20% of the transcriptome was modulated at least 2-fold after 48-hr lapatinib treatment in SKBR-3 and BT474 cells (Figure 2D). Gene ontology terms enriched in commonly upregulated genes involve regulation of glucose homeostasis and transcription, consistent with a reactivation of AKT signaling networks and reorganization of a significant portion of the transcriptome (Figure 2E). Kinases commonly upregulated 2-fold or more between SKBR-3 and BT474 cells include RTKs *INSR*, *ERBB3*, and *ERBB4*, the cytoskeleton-regulating kinases *TBCK*, *DCLK2*, and *TTBK2* and *DYRK1B*—a modulator of FOXO transcription (Figure 2F). *ERBB2*, *PTK7*, and *DDR1* in SKBR-3 and *EPHA7*, *MERTK*, *EPHA4*, *EGFR*, *IGF1R*, and *FGFR2* in BT474 are also upregulated, consistent with a transcriptional component of the adaptive RTK response. In BT474 cells, *IRS2* and *IGF1* (both 16-fold) are among the top 20 upregulated genes, indicating an autocrine/paracrine feedforward loop activating IGF1R signaling.

Significant Heterogeneity Exists among Kinases that Compensate for ERBB2 in the Presence of Lapatinib

Given the cluster of INSR/IGF1R activation, reports of IGF1R/ERBB2/ERBB3 complexes in trastuzumab-resistant cells (Huang et al., 2010), and the enrichment of glucose signaling networks from the RNA-seq data, we investigated the role of IGF1R and INSR in bypassing ERBB2 inhibition. SKBR-3 and BT474 cells were treated with increasing doses of the INSR/IGF1R inhibitor BMS754807 (BMS754) in the presence or absence of lapatinib (Figure 3A). The combination of lapatinib + BMS754 causes a dose-dependent increase in AKT phosphorylation in SKBR-3 but a decrease in BT474 cells. Combinations of lapatinib and BMS754 had little effect on HCC1419 and HCC1954 signaling (Figure S3F), but in MDA361 cells BMS754 alone reduced phosphorylation of ERBB2, ERBB3, FAK, SFKs, and AKT S473 and in combination with lapatinib further inhibited AKT (Figure 3B). Crystal violet colony formation assays demonstrated the combination of lapatinib and BMS754 significantly inhibited growth of MDA361 cells, but resistant colonies persisted, and no major enhancement of lapatinib-induced growth inhibition was observed in other cell lines (Figure 3C). Thus,

Figure 1. Lapatinib Induces Dynamic Kinome Responses

(A) Flowchart for experimental design.

(B) MIB/MS kinome activation dynamics over 48 hr of 300 nM lapatinib treatment in SKBR-3 cells. Ratios greater than 1 indicate increased MIB binding (increased activity), and values <1 indicate decreased MIB binding (decreased activity) relative to control cells treated with DMSO. Data presented are the average of four biological replicates.

(C) MIB-binding dynamics suggest reactivation of ERBB2 in SKBR-3 cells but continued suppression in BT474 cells.

(D) Hierarchical clustering of MIB-binding ratios in SKBR-3 cells identifies clusters of dynamic kinase behavior.
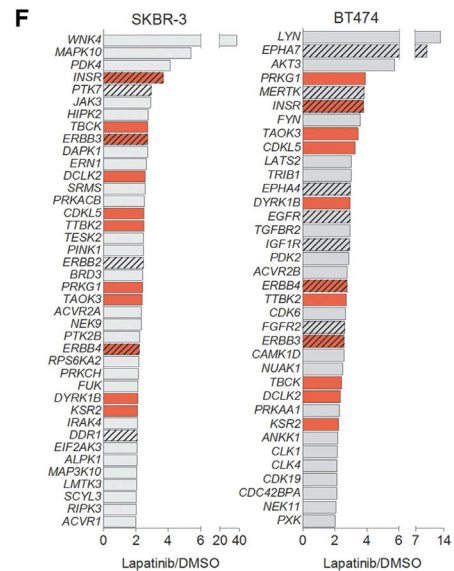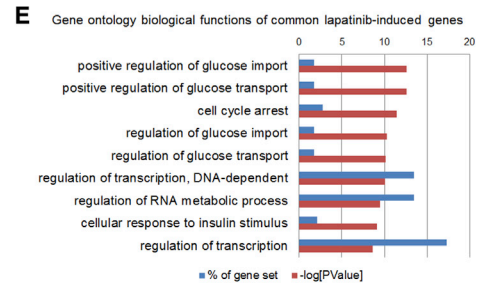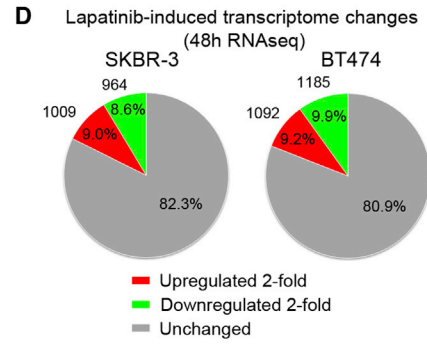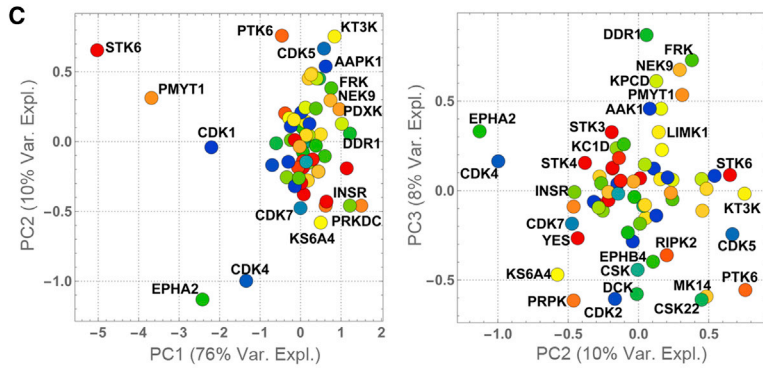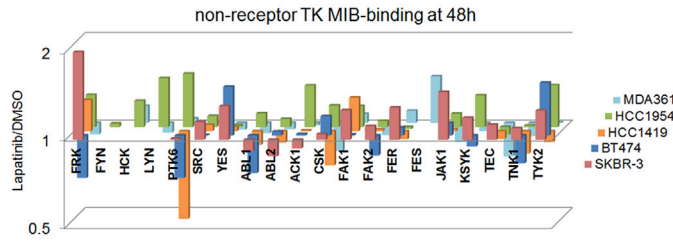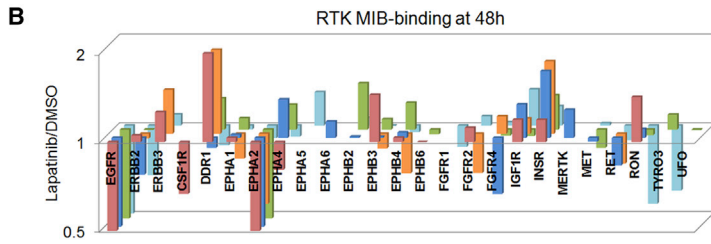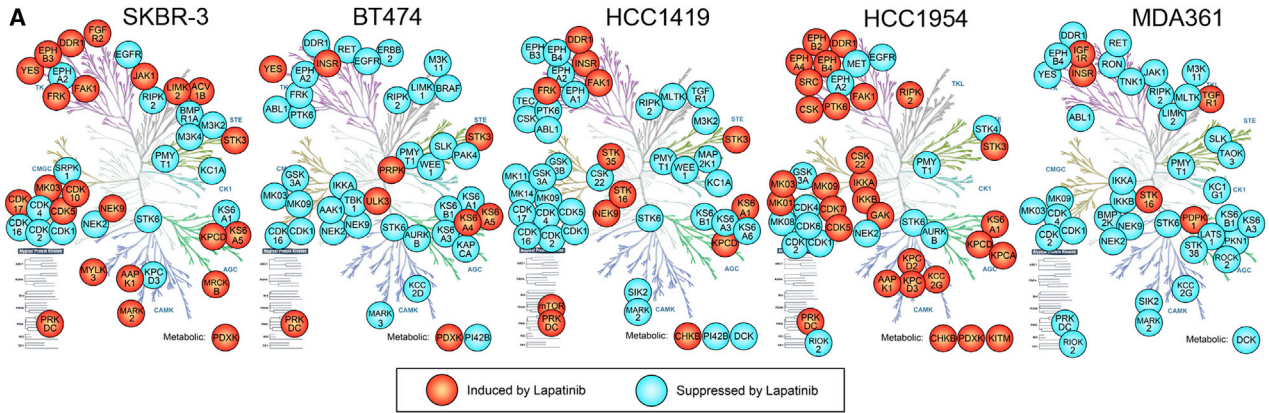
(E) Dynamics of a select set of kinases illustrates multiple behaviors in response to lapatinib. Four points graphically indicate 0, 4, 24, and 48 hr MIB binding. Blue, inhibited; red, activated.

(F) Western blots validate MIB/MS results and identify upregulation of ERBB3, DDR1, FRK, and PKCδ and increased activation of FAK, SRC family kinases (SFKs), and STAT3 in response to lapatinib. AKT and ERK1/2 are inhibited at 4 hr but become reactivated over 72 hr. Treatment with 1 μM lapatinib re-inhibits EGFR and ERBB2 but has little effect on other kinases.

(G) BT474 MIB-binding dynamics of tyrosine kinases and MEK/ERK and AKT/mTOR pathways.

(H) Western blots indicate upregulation of ERBB3, INSR, and IGF1R total levels and increase in SFK phosphorylation after lapatinib treatment in BT474 cells. Also see Figure S1.

A SKBR-3 BT474 HCC1419 HCC1954 MDA361

Induced by Lapatinib   Suppressed by Lapatinib

B RTK MIB-binding at 48h

non-receptor TK MIB-binding at 48h

MDA361
HCC1954
HCC1419
BT474
SKBR-3

C

D Lapatinib-induced transcriptome changes
(48h RNAseq)

SKBR-3   BT474

964   1185
1009   8.6%   1092   9.9%
9.0%   9.2%
82.3%   80.9%

Upregulated 2-fold
Downregulated 2-fold
Unchanged

E Gene ontology biological functions of common lapatinib-induced genes

positive regulation of glucose import
positive regulation of glucose transport
cell cycle arrest
regulation of glucose import
regulation of glucose transport
regulation of transcription, DNA-dependent
regulation of RNA metabolic process
cellular response to insulin stimulus
regulation of transcription

% of gene set   -log[PValue]

F SKBR-3   BT474

(legend on next page)

co-targeting ERBB2 and the common IGF1R/INSR response does not provide a successful pan combination therapy.

Lapatinib induces activity and transcription of DDR1, SFKs, and EPH receptors in multiple cell lines (Figures 2A, 2B, and 2F), all of which are targets for dasatinib, and previous reports link SFKs to escape from ERBB2-targeted therapies (Rexer et al., 2011; Zhang et al., 2011). Treatment of SKBR-3 and BT474 cells with dasatinib caused a loss of MIB binding of multiple RTKs and TKs and prevented lapatinib-induced MIB binding (Figure 3D). Colony formation assays demonstrated dasatinib only modestly enhances lapatinib growth inhibition in BT474 and MDA361 cells (Figure 3C). Western blots indicated the combination of lapatinib and dasatinib inhibits activation of SFKs, AKT, and ERK1/2 but actually increases FAK phosphorylation (Figure 3E). Lapatinib induced FAK MIB binding in multiple cell lines (Figure 2A), and the FAK inhibitor PF573228 in combination with lapatinib synergized in colony formation assays in SKBR-3 cells (Figure 3C). PF573228 inhibited FAK and SFK phosphorylation in the presence or absence of lapatinib (Figure 3F), suggesting both pathways must be inhibited in the presence of lapatinib to generate stable growth inhibition. While other cell lines do not demonstrate such strong synergism seen with SKBR-3 cells, FAK signaling is crucial for the growth of several cell lines and FAK inhibition enhances lapatinib growth inhibition.

FGFR2 was induced by lapatinib by MIB/MS (SKBR-3, Figure 2A) and RNA-seq (BT474, Figure 2F), and FGFR2 has been implicated in compensating for ERBB2 in the presence of lapatinib (Azuma et al., 2011), so we tested lapatinib + a pan-FGFR inhibitor (BGJ398). Colony formation assays demonstrated FGFR inhibition enhances lapatinib growth inhibition and SKBR-3 cells display moderate synergism between lapatinib and BGJ398 (Figure 3C). Most lines are growth-inhibited by BGJ398 in the absence of lapatinib, suggesting FGFRs cooperate with ERBB2 for growth of ERBB2$^+$ cells. Combining lapatinib and BGJ398 inhibited ERBB2/ERBB3 reactivation and further inhibited SFK and FAK phosphorylation in SKBR-3 cells but, in turn, elicited a stronger AKT/ERK response than lapatinib alone (Figure 3G). MIB/MS analysis of SKBR-3 cells demonstrated BGJ398 inhibits lapatinib induction of SFKs, FAK1/2, and multiple other TKs consistent with FGFR participation in lapatinib-induced kinome adaptation (Figure 3H). The combination of lapatinib and BGJ398 still allowed resistant colony formation in all five cell lines, suggesting alternative growth-promoting signaling networks are activated even in response to combined ERBB/FGFR/SFK/FAK inhibition. Overall, these results identify

multiple kinases that contribute to ERBB2$^+$ cell growth and reveal heterogeneity in the kinases that compensate for lapatinib-mediated ERBB2 inhibition.

Lapatinib-Resistant SKBR-3 and BT474 Depend on Multiple Kinases for Growth

To further define how the kinome bypasses inhibition of ERBB2, we generated lapatinib-resistant (LapR) SKBR-3 by continuous treatment with 300 nM lapatinib for 4+ months and LapR BT474 by progressively increasing doses of lapatinib every 3–4 weeks to 300 nM. Resistant lines were kept as a pool of all clones that grew out. LapR SKBR-3 grow at a similar rate to parental cells, while LapR BT474 grow somewhat slower than parental (Figures S4A and S4B). LapR cells were less sensitive to growth inhibition by ERBB2 knockdown but similarly sensitive to ERBB3 knockdown as compared to parental cells (Figure 4A). MIB/MS was used to compare LapR cells to lapatinib-sensitive parental cells (Figure S4C). Longtail plots of activated kinases show the most-activated kinases in LapR cells overlap with 48 hr MIB/MS signatures and transcriptome responses of the parental line and other ERBB2$^+$ lines (Figures 2A, 2F, and 4B). LapR SKBR-3 and BT474 cells share several activated kinases, including PRKDC, CDK5, TGFR1, ACVR1, CK1/2, and TKs MET, DDR1, FGFR2, FRK, and FER. Among RTKs, LapR SKBR-3 display strong activation of ERBB3 and modest increases in DDR1, FGFR2, and MET, while LapR BT474 activate multiple FGFRs, EPHA7, MERTK, MET, and IGF1R (Figure 4C). Western blots indicated inhibition of EGFR/ERBB2 phosphorylation in LapR cells with upregulation of multiple RTKs and reactivation of AKT/ERK in SKBR-3 cells but reduced activity of AKT/ERK in BT474 relative to parental cells (Figure 4D). Knockdown of ERBB RTKs, FGFR2, DDR1, MET, and CDK5 all provided partial growth inhibition of LapR SKBR-3 cells, while LapR BT474 cells are growth-inhibited by ERBB3, FGFR2, and CDK5 knockdown (Figures 4E and S4D–S4F). Thus, prolonged exposure to lapatinib causes a broad reorganization of the kinome and shifts dependency away from ERBB2 and toward multiple other kinases including several RTKs.

BET Family Bromodomain Inhibition Suppresses Lapatinib-Induced Kinome Reprogramming

By undertaking this comprehensive analysis, we unveiled significant heterogeneity in lapatinib-induced kinome adaptation in ERBB2$^+$ cells and demonstrated the resiliency of the kinome to bypass combinations of lapatinib and a second kinase inhibitor.

**Figure 2. MIB/MS and RNA Sequencing Define Heterogeneity in the Adaptive Response**

(A) Statistically significant MIB-binding changes after 48-hr lapatinib treatment based on Benjamini-Hochberg adjusted p values at FDR of 0.05 and SD in five ERBB2$^+$ cell lines depicted graphically. Kinome trees reproduced courtesy of Cell Signaling Technology.

(B) Lapatinib-induced MIB-binding changes of tyrosine kinases illustrate differences between cell lines and identifies common response of INSR and IGF1R activation.

(C) Principal component analysis identifies kinases that drive the variation in kinome response. Kinases captured in at least three out of four MIB/MS runs per cell line (67 kinases) were used.

(D) Lapatinib induces 2-fold changes up or down in 18%–20% of expressed mRNA transcripts.

(E) Gene ontology terms enriched in commonly upregulated mRNAs between SKBR-3 and BT474 cells identify glucose regulation and transcriptional regulation as most significant processes.

(F) Kinase mRNAs upregulated by lapatinib at least 2-fold by RNA-seq in SKBR-3 and BT474. Hatched bars indicate RTKs, and red bars indicate common upregulated kinases.

Also see Figures S2 and S3.
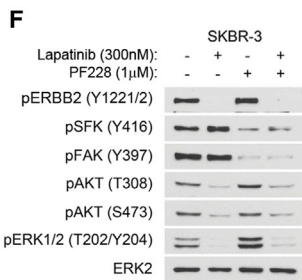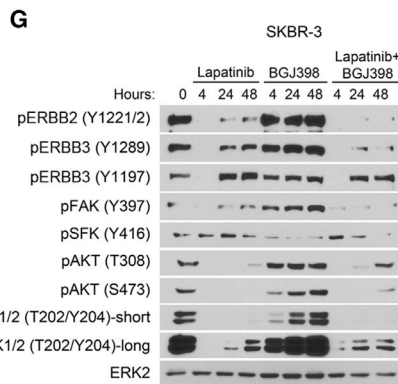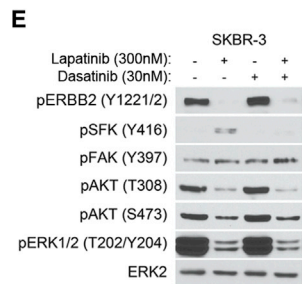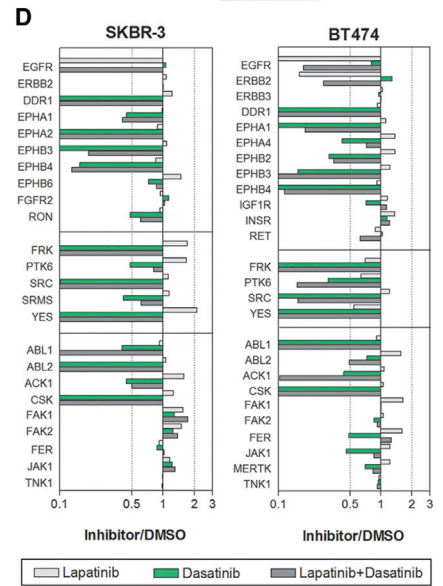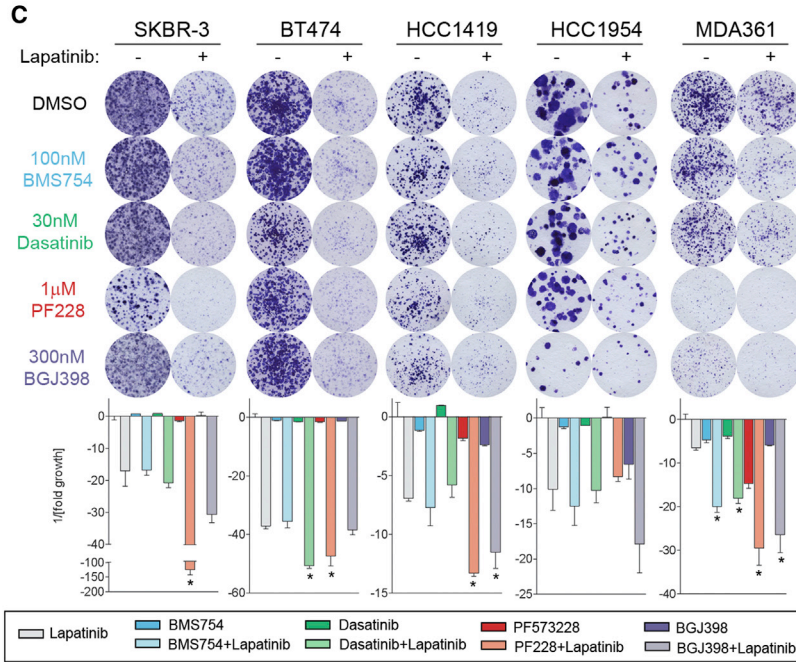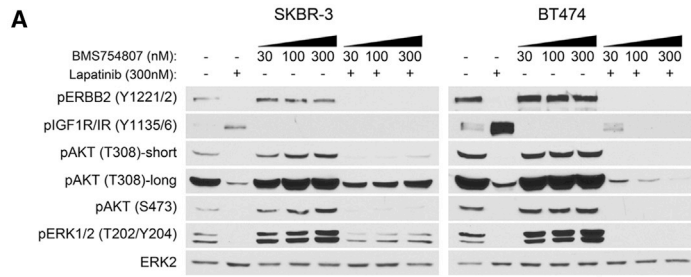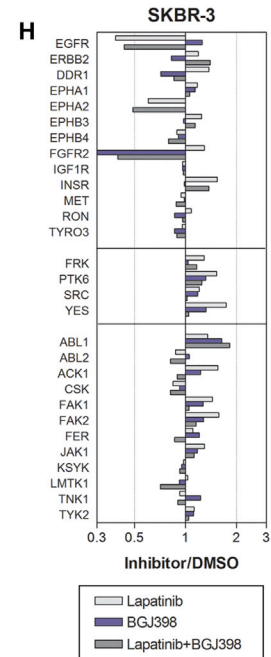
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This argues multiple sequential combinations of kinase inhibitors and possibly intermittent therapies might be necessary to prevent resistance, but rationally choosing such a regimen poses a significant challenge. Multiple kinases contribute to growth, and since there is no one drug that can inhibit the activity of all responsive kinases, we hypothesized that targeting the adaptive response itself would make lapatinib-induced growth arrest more durable (Figure 5A). RNA-seq analysis indicated 8%–10% of the expressed transcriptome is upregulated ≥2-fold within 48 hr of lapatinib treatment (Figure 2D). Of significance, kinases involved in resistance were transcriptionally induced by lapatinib treatment (e.g., ERBB3, DDR1, FGFR2), as were many kinases in the 48-hr MIB/MS signature of SKBR-3 cells (Figure S5A). This is consistent with lapatinib inhibiting AKT and ERK signaling networks causing FOXO activation and c-Myc degradation, leading to RTK upregulation (Chandarlapaty et al., 2011; Duncan et al., 2012). Thus, we decided to target epigenetic factors—proteins that modify or associate with chromatin—to prevent the reprogramming response at a transcriptional level. We tested inhibitors of different epigenetic enzymes and identified JQ1, an inhibitor of BET family bromodomains (Delmore et al., 2011) as capable of suppressing lapatinib-induced kinome reprogramming.

ERBB2$^+$ cell lines were sensitive to growth inhibition by JQ1 and I-BET762, a second BET bromodomain inhibitor (Figure S5B). Treatment of SKBR-3 cells with JQ1 prevented lapatinib-induced phosphorylation and expression of ERBB3, a primary mediator of the adaptive response leading to AKT reactivation and lapatinib resistance (Figure 5B). JQ1 also suppressed lapatinib-induced expression of FGFR2, DDR1, IGF1R, pFAK, pSFK, and pPKCδ across multiple cell lines (Figure 5C). JQ1 alone has little effect on AKT and p70 S6K phosphorylation but in combination inhibits the activity of both kinases more than that seen with lapatinib alone. Increased PARP cleavage was observed with the combination of lapatinib and JQ1 versus single agents, indicating an increase in apoptosis (Figure 5C). JQ1 also inhibited lapatinib-mediated kinase induction in HCC1954 and MDA361 cells, including growth-promoting kinases FGFR1, FGFR2, and IGF1R (Figure S5C). Treatment of SKBR-3 cells with another BET inhibitor, I-BET151, similarly blocked lapatinib-induced expression and phosphorylation of signature kinases (Figure S5D). Quantitative RT-PCR (qRT-PCR) analysis demonstrated JQ1 suppresses or prevents lapatinib-induced transcription of many adaptive response kinases implicated in resistance, including ERBB3, DDR1, FGFR2, IGF1R, and MET, in addition to ERBB2 itself (Figures 5D and S5E).

JQ1 alone only reduced the growth of SKBR-3 and BT474 cells and resulted in the formation of resistant colonies in 4-week clonogenic assays but when combined with lapatinib strongly arrested growth or resulted in regression of cell number and essentially eliminated colony formation (Figures 5E and 5F). I-BET762 and I-BET151 elicit similar growth-inhibitory responses from SKBR-3 and BT474 cells (Figures S6A–S6C). Clonogenic assays with HCC1419 cells demonstrated combinations of lapatinib, and BET bromodomain inhibitors suppressed ERBB2$^+$ cell growth more effectively than kinase inhibitor combinations with lapatinib (dasatinib, BMS754, PF228, and BGJ398) (Figure 5G). Growth of HCC1954 and MDA361 cells, which are more resistant to lapatinib than SKBR-3 or BT474 cells, was also inhibited by lapatinib + BET bromodomain inhibitors in 8-day and 5-week growth assays (Figures S6D–S6F).

Since AKT is a convergent node downstream of many RTKs and crucial to ERBB2$^+$ cell growth, we compared BET bromodomain inhibitors to AKT inhibitors in combination with lapatinib. In 8-day treatments of BT474 cells, the AKT inhibitor MK2206 alone or in combination with lapatinib induced multiple RTKs (ERBB3, DDR1, IGF1R, FGFR2) and resulted in increased FAK, SFK, and ERK phosphorylation (Figure 5H). Importantly, JQ1 or I-BET151 alone was unable to completely suppress signature kinase expression and signaling and only when combined with lapatinib inhibited RTK expression and activity and caused a loss of downstream FAK, SFK, AKT, ERK, and p70 S6K signaling (Figures 5H and S6G). This strongly suggests JQ1 inhibits reactivation of oncogenic signaling by suppressing the adaptive kinome response. 4-week growth assays indicated that while AKT inhibitors (MK2206 and GSK690693) work well in combination with lapatinib in BT474 cells, resistant colonies still

Figure 3. Cell Lines Exhibit Variability in Kinases that Drive Growth in the Presence and Absence of Lapatinib

(A) Lapatinib combined with increasing doses of BMS754807 (IGF1R/INSR inhibitor) causes an increase in AKT phosphorylation in SKBR-3 but a decrease in BT474 relative to lapatinib treatment alone after 24 hr.

(B) BMS754 inhibits ERBB2/3 phosphorylation as a single agent, and when combined with lapatinib causes a further inhibition of AKT phosphorylation after 24 hr.

(C) Colony formation assays indicate heterogeneity in the kinases that contribute to growth. IGF1R/INSR inhibition has an additive effect with lapatinib in MDA361. Dasatinib is additive in BT474 and MDA361 but does not significantly enhance growth inhibition of other lines. SKBR-3 cells display synergism between lapatinib and PF228 (FAK inhibitor) or BGJ398 (FGFR inhibitor), but other cell lines show varying degrees of growth inhibition by FAK or FGFR inhibition alone and in combination with lapatinib. SKBR-3, BT474, and HCC1419 were treated for 4 weeks, HCC1954 and MDA361 were treated for 5 weeks. Lapatinib doses: 100 nM SKBR3; 30 nM BT474; 10 nM HCC1419; 300 nM HCC1954 and MDA361. Data presented are mean ± SD of three technical replicates. *Significant difference from lapatinib alone (p < 0.05).

(D) MIB/MS profile of SKBR-3 and BT474 cells after 48-hr treatment with 300 nM lapatinib, 30 nM dasatinib, or a combination. Dasatinib inhibits MIB binding of multiple tyrosine kinases, but not FAK1 and FAK2 in SKBR-3 cells.

(E) Western blots after 48 hr demonstrate dasatinib inhibits lapatinib-induced SFK phosphorylation but increases FAK phosphorylation.

(F) Western blots after 48 hr indicate PF228 inhibits FAK and SFK phosphorylation but increases AKT and ERK1/2 phosphorylation.

(G) Western blots indicate FGFR inhibition alone slightly reduces AKT and ERK phosphorylation at 4 hr but elicits strong reactivation by 48 hr. Combination with lapatinib indicates FGFRs regulate ERBB signaling and SFK and FAK phosphorylation. 300 nM lapatinib and 300 nM BGJ398 added directly to media at 0 hr. Media were not changed throughout experiment.

(H) MIB/MS analysis of 300 nM lapatinib, 300 nM BGJ398, or the combination after 48 hr indicates FGFRs regulate multiple lapatinib-induced TKs.

(I) Matrix of p values comparing growth inhibition of lapatinib alone versus lapatinib + kinase inhibitor in colony formation assays. Red, significant (p < 0.05); blue, not significant (p ≥ 0.05).
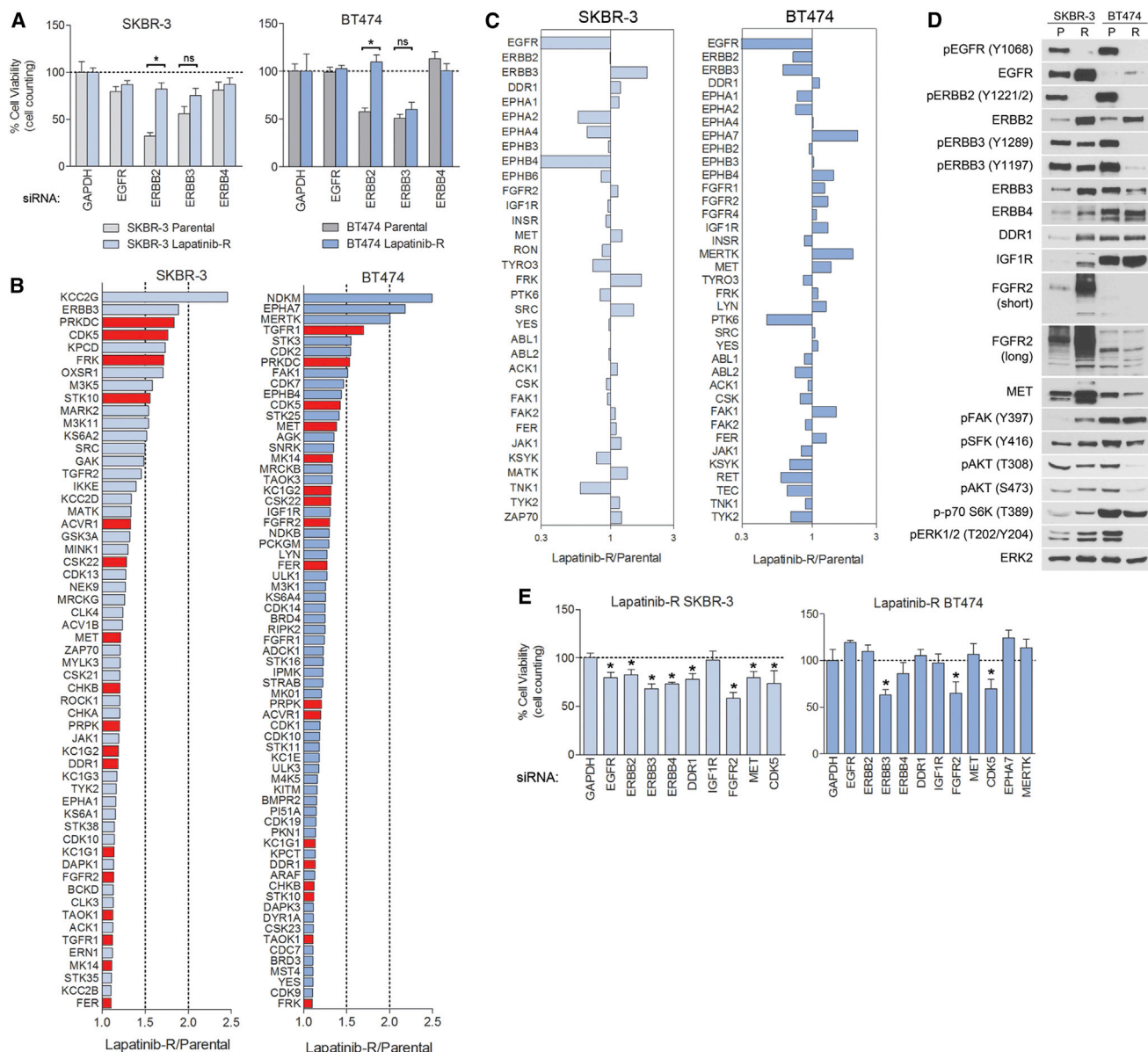
Figure 4. Multiple Unrelated Kinases Contribute to the Growth of Lapatinib-Resistant Cells

(A) Parental or 300 nM lapatinib-resistant (LapR) SKBR-3 and BT474 cells were transfected with small interfering RNAs (siRNAs) against GAPDH (control) or ERBB receptors and cultured for 96 hr. Both parental lines are strongly growth inhibited by ERBB2 and ERBB3 knockdown. LapR cells are less dependent on ERBB2 but remain similarly dependent on ERBB3.

(B) MIB/MS long tail plots of most-activated kinases in LapR SKBR-3 and BT474 cells, relative to parental cells. Kinases in red are commonly over-activated in SKBR-3 and BT474.

(C) MIB/MS profile of tyrosine kinases from LapR SKBR-3 and BT474 cells. LapR SKBR-3 cells display enhanced MIB binding of ERBB3, DDR1, FGFR2, MET, FRK, and SRC. LapR BT474 have increased activity of multiple FGFRs, EPHA7, IGF1R, MERTK, MET, LYN, and FAK1. Data presented are mean of two biological replicate MIB/MS experiments.

(D) Western blots indicate RTK upregulation in LapR SKBR-3 cells and reactivation of AKT/ERK signaling. LapR BT474 cells display suppressed activity of AKT and ERK relative to parental cells. P, parental; R, LapR.

(E) 96-hr siRNA knockdown in LapR SKBR-3 cells indicates slight dependency on ERBB family and a stronger dependency on DDR1, FGFR2, and CDK5. BT474 cells are growth-inhibited by ERBB3, FGFR2, and CDK5 knockdown.

Data presented in (A) and (E) are mean ± SD of six technical replicates. Also see Figure S4.

form in SKBR-3 and HCC1419 cells (Figure S6H). This contrasts with the lack of colony formation in lapatinib + JQ1 combinations across all lines. These findings further support disruption of AKT/ERK signaling networks leading to RTK upregulation and a sustained blockade of the adaptive response by BET bromodomain inhibition.
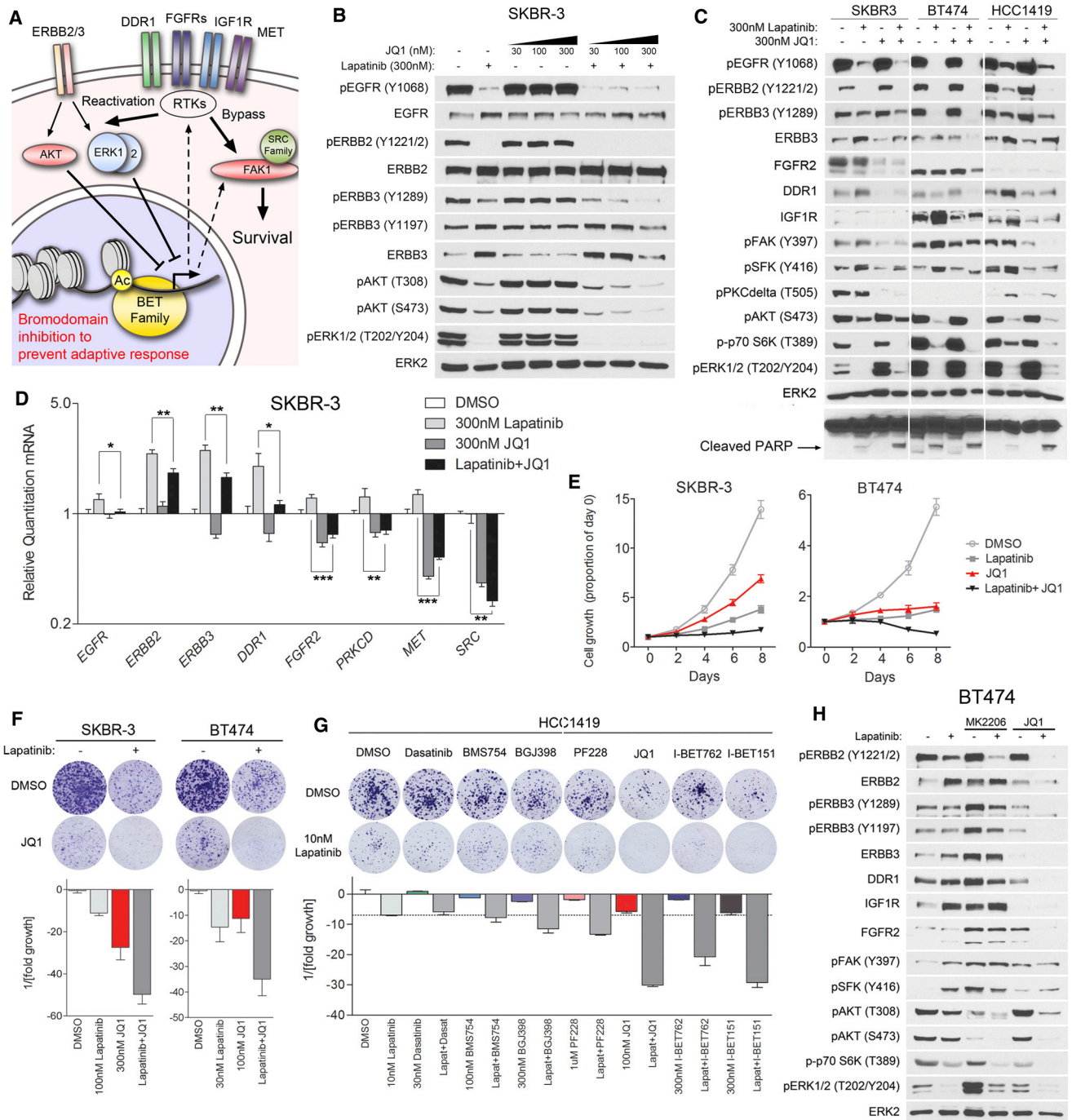
Figure 5. BET Bromodomain Inhibition Suppresses Lapatinib-Induced Kinome Reprogramming and Arrests Growth

(A) Kinome reprogramming leads to transcriptional upregulation of multiple alternative kinases capable of reactivating or bypassing ERBB2-directed signaling. We hypothesize by inhibiting the BET family of bromodomain-containing acetylation readers, we can prevent the adaptive response at an epigenetic level.

(B) Western blots demonstrate JQ1 (BET family bromodomain inhibitor) suppresses lapatinib-induced ERBB3 phosphorylation and expression at 300 nM and inhibits reactivation of AKT in SKBR-3 cells. 48-hr treatments.

(C) Western blots indicate JQ1 blocks protein expression of multiple kinases involved in lapatinib resistance and leads to a decrease in ERBB family, SFK, FAK, and PKCδ phosphorylation. JQ1/lapatinib combinations inhibit AKT and p70 S6K phosphorylation more than lapatinib alone and increase cleavage of PARP. 48-hr treatments.

(D) qRT-PCR after 24-hr treatment shows JQ1 inhibits mRNA transcription of multiple RTKs involved in resistance (*ERBB3*, *DDR1*, *FGFR2*, *MET*) and suppresses lapatinib-mediated induction.

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JQ1 Regulates Lapatinib-Induced Transcription and Releases BRD4 and pSer2-Polymerase II from Adaptive Kinase Genes

RNA-seq analysis demonstrated JQ1 downregulates 8% and 11% of genes at least 2-fold in SKBR-3 and BT474 cells, respectively (Figures 6A and S7A). A smaller percentage was upregulated 2-fold, with 3% in SKBR-3 and 5% in BT474, indicating JQ1 affects transcription overall less than lapatinib. In combining lapatinib and JQ1, we found that genes upregulated by lapatinib were suppressed by JQ1 more than those unaffected or downregulated by lapatinib (Figures 6B–6E and S7B–S7E). Of transcripts induced at least 2-fold by lapatinib in SKBR-3 cells, adding JQ1 suppressed the induction of 27% by at least half, and further upregulated just 4% (Figure 6C). Similarly, in BT474 cells JQ1 suppressed 28% of lapatinib-induced genes (Figure S7C). This indicates that JQ1 preferentially modulates lapatinib-responsive gene expression. Reports indicate suppression of *MYC* transcription is central to JQ1 function. BT474 cells stably overexpressing c-Myc were less sensitive to JQ1, but the combination of lapatinib + JQ1 still caused growth arrest in SKBR-3 and cell regression in BT474 despite the rescue of c-Myc levels (Figures S7F and S7G).

RNAi was used to inhibit the expression of BET family members BRD2, -3, or -4 in SKBR-3 and BT474 cells (Figures 6F, S7H, and S7I). BRD2 and BRD3 knockdown actually increased target kinase transcription in response to lapatinib. In contrast, lapatinib in combination with BRD4 knockdown was similar to JQ1 in reducing lapatinib-mediated induction of *ERBB3* and *DDR1* expression. Chromatin immunoprecipitation (ChIP)-PCR demonstrated that JQ1 displaces BRD4 from the promoters and upstream elements of lapatinib-induced kinase genes *ERBB2*, *ERBB3*, *FGFR2*, and *DDR1* (Figures 6G and S7J). *KIT* is not expressed in SKBR-3 cells and serves as a negative control for ChIP-PCR analysis. JQ1 treatment also reduces the level of the elongating form of RNA Polymerase II (phospho-Serine 2 of the C-terminal tail repeat; pS2-Pol2) binding to promoters and internal exons of target kinase genes, consistent with transcriptional inhibition (Figures 6G and S7J). Importantly, BRD4 and pS2-Pol2 are most effectively dissociated from chromatin by combined lapatinib + JQ1 treatment, indicating synergism between these drugs at an epigenetic level.

BET Bromodomain Inhibition Re-sensitizes Resistant Cells to Lapatinib

RNA-seq of LapR SKBR-3 cells following 8-day treatment with combinations of lapatinib with JQ1 or I-BET151 indicated transcriptional suppression of a large proportion of TKs, including those that contribute to growth (*ERBB3*, *DDR1*, *FGFR2*, and *MET*; Figure 7A). Many outlier kinases from PCA (a representation of heterogeneity in the kinome response; Figure 2C) were also suppressed by BET bromodomain inhibition (Figure 7B). MIB/MS analysis from the same treatments indicated JQ1 and I-BET151 inhibit or block the activity of the most-induced kinases in LapR SKBR-3 cells relative to parental cells (Figure 7C). Accordingly, JQ1 and I-BET151 inhibited the protein expression and phosphorylation of signature kinases in LapR SKBR-3 and BT474 cells, effectively reversing the adaptive kinome reprogramming response (Figure 7D). Four-week clonogenic growth assays demonstrated that the combination of lapatinib and JQ1 arrested the growth of lapatinib-resistant cells (Figure 7E). Furthermore, combinations of lapatinib and BET bromodomain inhibitors were superior to combinations of lapatinib and kinase inhibitors that only slowed the growth of LapR cells (Figure 7F). Lapatinib + BET bromodomain inhibitor combinations were even significantly more effective than the triple combination of lapatinib, dasatinib, and FAK inhibitor. This indicates that arresting the transcriptional reprogramming response is more effective than inhibiting the activity of multiple induced kinases. While BET inhibitors arrested the growth of LapR cells in combination with lapatinib, removal of lapatinib from the media while maintaining JQ1 or I-BET762 in the culture allowed the cells to begin growing again (Figure 7G). Thus, lapatinib and BET bromodomain inhibitor must be present in combination to effectively inhibit growth; JQ1 or I-BET alone is not sufficient to inhibit growth of the cells. Since LapR BT474 cells did not depend on EGFR or ERBB2 for growth in the presence of lapatinib (Figure 4A), this indicates BET bromodomain inhibitors sensitize cells to lapatinib by blocking alternative kinases involved in adaptive growth responses.

DISCUSSION

In this study, we used MIB/MS to define lapatinib-induced kinase activation dynamics on a kinome-wide level. This global approach unveiled a robust network of kinases that compensate for ERBB2 inhibition induced within 48 hr of lapatinib treatment, indicating multiple potential mechanisms of resistance emerge rapidly upon kinase inhibitor treatment. Inhibition of different induced kinases in combination with lapatinib increased growth inhibition across the five ERBB2^{+} cell lines to varying degrees. Strong growth inhibition was observed by targeting FAK in combination with lapatinib in SKBR-3 cells, indicating significant synergism can be achieved if such vulnerable nodes are defined. Heterogeneity in the adaptive kinome response, however, makes identifying effective combination inhibitor treatments a challenging task. Adding to the problem is the differential

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(E) 8-day growth curves demonstrate JQ1/lapatinib combination prevents growth of SKBR-3 and BT474 cells. Data presented are mean ± SD of six technical replicates.

(F) JQ1 in combination with lapatinib suppresses colony formation of SKBR-3 and BT474 cells after 4 weeks.

(G) BET family bromodomain inhibitors (JQ1, I-BET762, I-BET151) suppress colony formation of HCC1419 cells more so than kinase inhibitors in combination with lapatinib in 4-week colony formation assays.

(H) Western blots indicate AKT inhibition (MK2206) induces RTK expression and ERK signaling alone and in combination with lapatinib. BET bromodomain inhibition alone does not sustain inhibition of signature kinases, and only in combination with lapatinib suppresses the adaptive response. 8-day treatment with 30 nM lapatinib, 100 nM MK2206, and 300 nM JQ1.

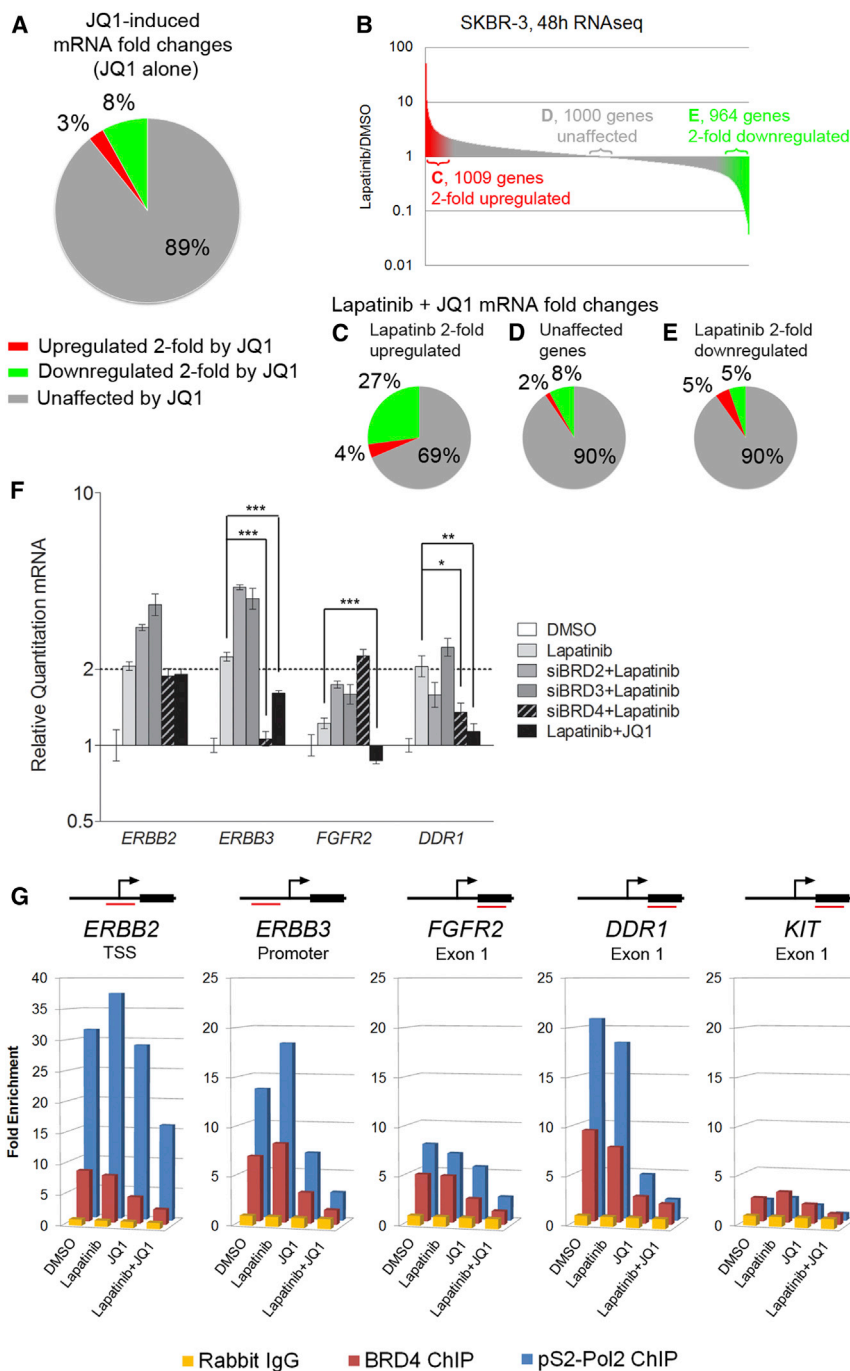Data presented in (D), (F), and (G) are mean ± SD of three technical replicates. Also see Figures S5 and S6.

A JQ1-induced mRNA fold changes (JQ1 alone)

- Upregulated 2-fold by JQ1
- Downregulated 2-fold by JQ1
- Unaffected by JQ1

B SKBR-3, 48h RNAseq

Lapatinib + JQ1 mRNA fold changes

C Lapatinib 2-fold upregulated

D Unaffected genes

E Lapatinib 2-fold downregulated

F

G

- Rabbit IgG
- BRD4 ChIP
- pS2-Pol2 ChIP

Figure 6. JQ1 Modulates Lapatinib-Induced Transcription and Inhibits Epigenetic Regulation of Signature Kinase Genes

(A) RNA-seq indicates JQ1 affects 11% of expressed genes 2-fold or more in SKBR-3 cells after 48-hr treatment.

(B) Figures 6C–6E refer to JQ1 effect on lapatinib-regulated genes as indicated.

(C) JQ1 downregulates 27% of the 1,009 lapatinib-induced genes by at least 2-fold from the lapatinib-induced mRNA level.

(D) 1,000 genes not affected by lapatinib treatment display a similar up- or downregulation profile in the lapatinib+JQ1 combination compared to JQ1 alone.

(E) 964 genes at least 2-fold downregulated by lapatinib are mostly unaffected by JQ1 as compared to JQ1 alone or JQ1 effects on lapatinib-upregulated genes.

(F) qRT-PCR demonstrates siRNA-mediated knockdown of BRD2 and BRD3 enhances transcription of *ERBB2*, *ERBB3*, *FGFR2*, and *DDR1*. Knockdown of BRD4 suppresses *ERBB3* and *DDR1* transcription, similar to JQ1. 24-hr siRNA knockdown and then 24-hr drug treatment; 300 nM JQ1, 300 nM lapatinib. Data presented are mean ± SD of three technical replicates.

(G) ChIP-PCR indicates JQ1 inhibits BRD4 promoter occupation in the absence of lapatinib. Loss of BRD4 and elongating RNA Polymerase II (pS2-Pol2) from upstream elements is maximal when JQ1 is combined with lapatinib. 4-hr treatments with 300 nM lapatinib and 300 nM JQ1 in SKBR-3 cells. Data presented are mean of three biological replicate experiments.

Also see Figure S7.

of ERBB2 inhibition, suggesting intrinsic resistance to ERBB2-targeted therapies can be rooted in dependence on multiple alternative kinases. Successful treatment of such tumors would be difficult with combinations of kinase inhibitors. Heterogeneity of kinase expression in different regions of the tumor would further enhance this dilemma. Our study demonstrates BET bromodomain inhibition provides an epigenetic mechanism to target a series of kinases that mediate resistance and sustain ERBB2⁺ cell growth. Indeed, MDA361 cells are the most sensitive to the JQ1/lapatinib combination treatment even though they are relatively insensitive to lapatinib alone.

dependence of tumor cells on unrelated kinases in addition to ERBB2. Together, these findings present a dilemma where combinations of any two or even three kinase inhibitors would be insufficient to suppress the resiliency of the kinome and sustain inhibition of tumor cell growth.

The five cell lines used in our study are each ERBB2⁺, but HCC1954 and MDA361 are less sensitive to lapatinib than the other three lines. MDA361 cells respond to inhibitors of IGF1R/INSR, SFKs, FAK, and FGFRs in the absence

We acknowledge inhibition of major epigenetic regulators such as BET bromodomain proteins has effects beyond the blockade of adaptive kinome reprogramming. Histone deacetylase inhibitors such as panobinostat are in clinical use and have been shown in melanoma to suppress resistance mechanisms to BRAF inhibition (Johannessen et al., 2013). We found panobinostat similarly blocks adaptive reprogramming in ERBB2⁺ breast
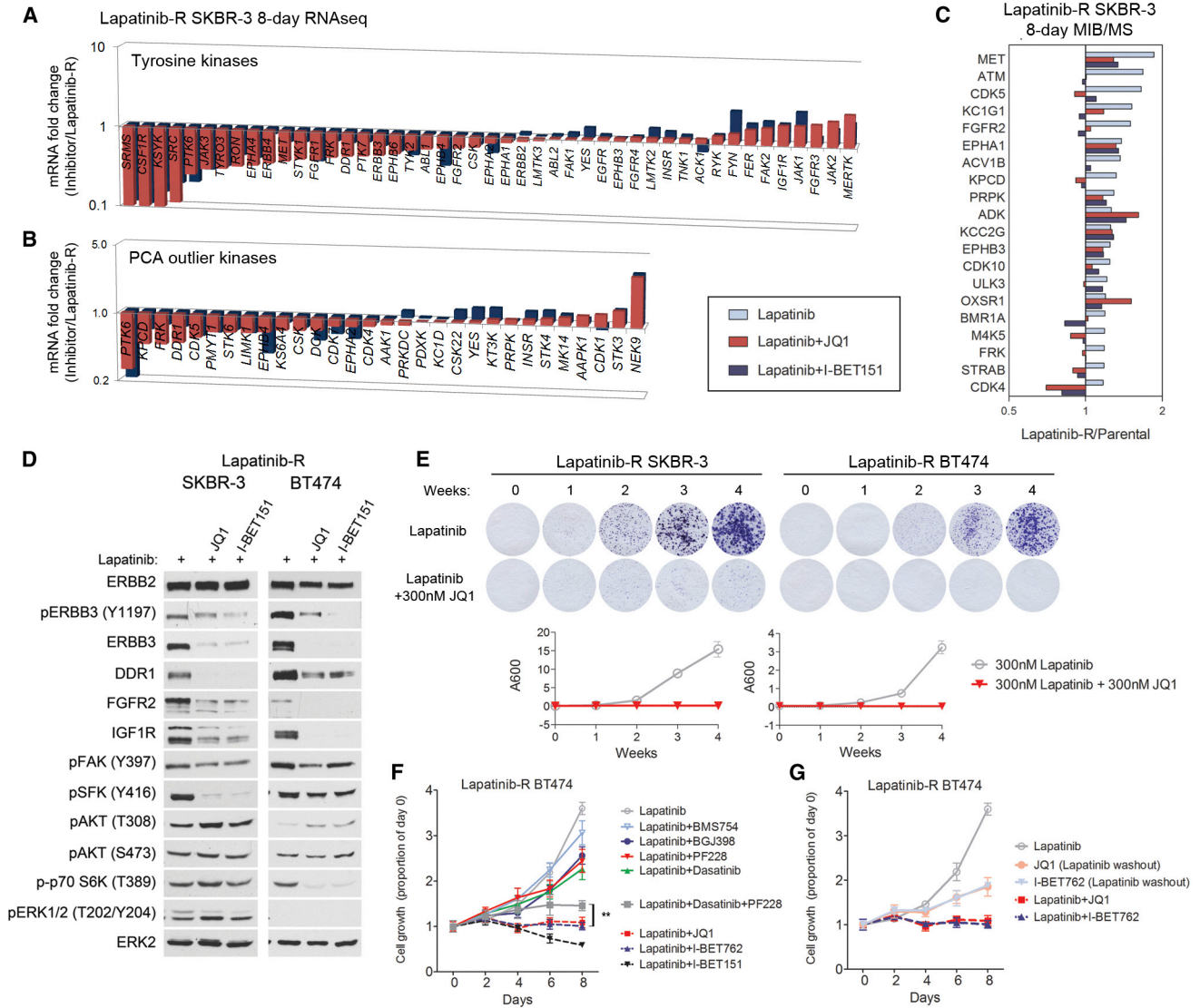
Figure 7. BET Bromodomain Inhibition Suppresses Signature Kinases and Arrests Growth in Lapatinib-Resistant Cells

(A) RNA-seq after 8-day treatment of lapatinib-resistant (LapR) SKBR-3 cells with lapatinib + 300 nM JQ1 or 1 μM I-BET151 indicates transcriptional suppression of the majority of tyrosine kinases.

(B) mRNA fold changes in outlier kinases identified by PCA (Figure 2C) indicates BET inhibitors suppress the majority of kinases that drive variation in the kinome response.

(C) MIB/MS analysis of the top 20 most-activated kinases in LapR SKBR-3 cells following 8 days treatment with 300 nM JQ1 or 1 μM I-BET151 indicates the majority of kinase activity is inhibited or blocked.

(D) Western blots of LapR SKBR-3 and BT474 cells treated with 300 nM JQ1 or 1 μM I-BET151 in combination with 300 nM lapatinib show suppression of signature kinase expression and phosphorylation.

(E) 4-week colony formation assays demonstrate JQ1 suppresses colony formation and arrests growth of LapR SKBR-3 and BT474 cells in the presence of lapatinib. Data presented are mean ± SD of three technical replicates.

(F) LapR BT474 cells are moderately growth-inhibited by combinations of lapatinib and other kinase inhibitors, but growth is completely suppressed by lapatinib and bromodomain inhibitors (300 nM JQ1, 1 μM I-BET762, or 1 μM I-BET151), even more effectively than a triple kinase inhibitor combination (lapatinib + dasatinib + PF228).

(G) Growth of LapR BT474 cells is arrested with 300 nM JQ1 or 1 μM I-BET762, but only in the presence of lapatinib.

Data presented in (F) and (G) are mean of six technical replicates ±SD.

cancer cells, but it also displays significant cellular toxicity in the absence of lapatinib. In contrast, we identified a significant molecular synergism between BET bromodomain inhibitors and lapatinib that inhibited RNA polymerase II function, kinase expression, and phosphorylation. The ChIP-PCR data with SKBR-3 and long-term signaling studies with BT474 indicate

the combination of lapatinib and BET bromodomain inhibitors is required to substantially suppress transcription of RTKs and prevent reactivation of AKT/p70 S6K signaling. These effects are not observed by JQ1 or I-BET151 treatment alone and suggest BET bromodomain inhibitors target the epigenetic machinery involved in the adaptive reprogramming response to lapatinib. RNA-seq indicates approximately 2,000 expressed genes are up or downregulated 2-fold or greater by lapatinib. This adaptive transcriptome response involves a global reorganization of signaling that is borne out by significant changes in kinome activation dynamics. This argues that targeting broad-acting epigenetic regulators of transcription like BET bromodomain proteins is not only advantageous, but needed to suppress this dramatic induction of gene expression. JQ1 suppresses 27% of lapatinib-induced transcripts by at least half, in contrast to 8% of genes as a whole. JQ1 thus has a selective inhibition of lapatinib-induced transcripts. Small interfering RNA (siRNA) experiments identified BRD4 as participating in the reprogramming response. BRD4 is a core component of the P-TEFb transcriptional elongation complex (Jang et al., 2005; Yang et al., 2005) and regulates the phosphorylation of RNA polymerase II for activation of transcriptional elongation of newly induced genes (Devaiah et al., 2012). Disruption of this complex by targeting BRD4 function provides an elegant mechanism of how BET bromodomain inhibition might regulate kinome reprogramming.

By targeting chromatin readers, we suppress expression of the majority of kinases having a potential role in lapatinib resistance and provide a method to address both the heterogeneity in kinome response and inhibit a broad panel of kinases known to drive ERBB2$^+$ cancer cell growth. Recent studies have described similar RTK networks comprised of ERBB receptors, MET, IGF1R, and FGFRs that become upregulated after targeted RTK inhibition (Singleton et al., 2013; Zhang et al., 2014). It is conceivable that BET bromodomain inhibition would suppress these kinases in other cancers as well and provide a means to block the adaptive response to EGFR and FGFR inhibition observed in these studies. We believe epigenetic enzyme-targeting drugs will be key to preventing resistance rooted in kinome reprogramming, thus making the action of kinase inhibitors durable. With at least four BET bromodomain inhibitors in clinical trials, testing of a BET bromodomain inhibitor to block adaptive responses induced with kinase inhibitors is a possibility.

EXPERIMENTAL PROCEDURES

MIB Chromatography and LC/MS

MIB preparation and chromatography was performed as previously described (Duncan et al., 2012). For multiplexing, peptides were labeled with iTRAQ and separated on a 288 or 300 min 5%–45% ACN gradient as a single fraction. ABSciex 5800 MALDI TOF/TOF and Thermo Q-Exactive ESI mass spectrometers were used. For details, see Supplemental Experimental Procedures.

For cell assays, statistics, western blotting, qRT-PCR, RNA-seq, and ChIP-PCR, see Supplemental Experimental Procedures.

ACCESSION NUMBERS

The SRA accession numbers for the mRNA sequencing data reported in this paper are found in Table S4.

AUTHOR CONTRIBUTIONS

T.J.S. and G.L.J. wrote the manuscript. D.A.G. performed qRT-PCR analysis of mRNA and kinase inhibitor coupling for MIBs. T.J.S. performed all other experiments. J.S.Z., A.S.B., J.S.P., and N.S. assisted RNA-seq analysis. J.S.Z. and K.N. assisted ChIP-PCR assay development. R.A.R. assisted mass spectrometry analysis. P.-F.K. performed statistical analyses of MIB/MS. X.C. and J.J. developed synthesis scheme for linker-adapted kinase inhibitors. S.M.G. and K.A.L.C. performed PCA. S.M.M., J.S.D., S.P.A., L.M.G., H.S.E., and L.A.C. contributed to experimental design.

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