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The Role of BPTF in Melanoma Progression and in Response to BRAF-Targeted Therapy

Altaf A. Dar, Mehdi Nosrati, Vladimir Bezrookove, David de Semir, Shahana Majid, Suresh Thummala, Vera Sun, Schuyler Tong, Stanley P. L. Leong, David Minor, Paul R. Billings, Liliana Soroceanu, Robert Debs, James R. Miller III, Richard W. Sagebiel, Mohammed Kashani-Sabet

Affiliations of authors: Center for Melanoma Research and Treatment (AAD, MN, VB, DdS, ST, VS, ST, SPLL, DM, JRMIII, RWS, MKS), California Pacific Medical Center Research Institute, San Francisco, CA (AAD, MN, VB, DdS, ST, VS, ST, SPLL, DM, LS, RD, JRMIII, RWS, MKS); Department of Urology, Veterans Affairs Medical Center and University of California San Francisco, San Francisco, CA (SM); Life Technologies, Inc. Carlsbad, CA (PRB).

Correspondence to: Mohammed Kashani-Sabet, MD, Center for Melanoma Research and Treatment, California Pacific Medical Center Research Institute, 475 Brannan St, Suite 220, San Francisco, CA 94107 (e-mail: kashani@cpmcri.org).

Abstract

Background: Bromodomain PHD finger transcription factor (BPTF) plays an important role in chromatin remodeling, but its functional role in tumor progression is incompletely understood. Here we explore the oncogenic effects of BPTF in melanoma.

Methods: The consequences of differential expression of BPTF were explored using shRNA-mediated knockdown in several melanoma cell lines. Immunoblotting was used to assess the expression of various proteins regulated by BPTF. The functional role of BPTF in melanoma progression was investigated using assays of colony formation, invasion, cell cycle, sensitivity to selective BRAF inhibitors, and in xenograft models of melanoma progression (n = 12 mice per group). The biomarker role of BPTF in melanoma progression was assessed using fluorescence in situ hybridization and immunohistochemical analyses. All statistical tests were two-sided.

Results: shRNA-mediated BPTF silencing suppressed the proliferative capacity (by 65.5%) and metastatic potential (by 66.4%) of melanoma cells. Elevated BPTF copy number (mean ≥ 3) was observed in 28 of 77 (36.4%) melanomas. BPTF overexpression predicted poor survival in a cohort of 311 melanoma patients (distant metastasis-free survival \(P = .03\), and disease-specific survival \(P = .008\)), and promoted resistance to BRAF inhibitors in melanoma cell lines. Metastatic melanoma tumors progressing on BRAF inhibitors contained low BPTF-expressing, apoptotic tumor cell subclones, indicating the continued presence of drug-responsive subclones within tumors demonstrating overall resistance to anti-BRAF agents.

Conclusions: These studies demonstrate multiple protumorigenic functions for BPTF and identify it as a novel target for anticancer therapy. They also suggest the combination of BPTF targeting with BRAF inhibitors as a novel therapeutic strategy for melanomas with mutant BRAF.
Epigenetic mechanisms, including post-translational modifications of histones, DNA methylation, incorporation of histone variants, and nucleosome remodeling have evolved to regulate the structure of chromatin and access to DNA. Nucleosome remodeling and the incorporation of histone variants are largely accomplished through the action of adenosine triphosphate (ATP)-dependent chromatin-remodeling complexes, which represent critical components of the machinery that controls gene expression. ATP-dependent chromatin-remodeling factors are classified into four major subfamilies (ISWI, SWI/SNF, CHD, and INO80) based upon sequence homology of the associated ATPase (1).

Nucleosome remodeling factor (NURF), initially identified in Drosophila melanogaster (2), is a key member of the ISWI family. The NURF301 homolog exists across all eukaryotic species and is evolutionarily conserved. The NURF complex mediates some of its cellular functions through interaction with sequence-specific transcription factors (3,4). NURF301 has two well-characterized domains that bind specific histone post-translational modifications. The PHD finger juxtaposed to the bromodomain interacts with H3K4me2/3, and the adjacent bromodomain binds H4K16ac (5,6). In addition, NURF likely interacts directly with DNA in a sequence-specific fashion (7).

In mammals, bromodomain PHD finger transcription factor (BPTF) represents the orthologue of NURF301, the largest subunit of the NURF chromatin remodeling complex (3). BPTF has been reported to be essential to embryonic development (4) and involved in ATP-dependent chromatin remodeling (8). Previously, we identified BPTF as statistically significantly overexpressed in metastatic melanomas by cDNA microarray analysis (9).

The human BPTF gene is located on chromosome 17q24, presumed to contain oncogenic elements given the demonstration of chromosomal gains in this locus in various tumors (10–13). FAC1 (Fetal Alz-50-reactive clone 1), a truncated form of BPTF, is upregulated in neurodegenerative diseases (14), and may function in transcriptional regulation (15). While the biological significance of BPTF to chromatin remodeling is clear (16,17), to date, its functional role in tumorigenesis is poorly understood. In this study, we examine the functional and biological roles of BPTF in melanoma.

Methods

RNA Extraction and Quantitative Real-Time Polymerases Chain Reaction

RNA extraction and cDNA synthesis were performed as described in Supplementary Methods (available online). mRNAs were assayed using the TaqMan Gene Expression Assays in accordance with the manufacturer’s instructions (Applied Biosystems, Foster City, CA) as described (18,19). TaqMan probes for BPTF, HPRT1, CCND2, BCL-XL, ERK1, ERK2, and BCL2 were purchased from Applied Biosystems.

Colony Formation, Cell Cycle Analyses, and BRAF Inhibitor Treatment

Assays for colony formation and cell cycle analysis are described in Supplementary Methods (available online). Cells were treated with varying concentrations of vemurafenib for 72 hours or dabrafenib (Chemietek, Indianapolis, IN) for 48 hours or as indicated. Dimethyl sulfoxide was used as a vehicle.

Animal Studies

All animal care was in accordance with institutional guidelines and a protocol that was approved by the University of California San Francisco Committee on Animal Research and the California Pacific Medical Center Research Institute. For tail vein injection 30,000 B16-F10 cells or 1x10^7 1205-Lu cells were injected in C57Bl/6 (44 days old, female) (n = 12) and nude mice (nu/nu, 44 days old, female) (n = 12) (Charles River, Wilmington, MA), respectively. For subcutaneous injection, 1x10^6 B16-F10, 1205-Lu, or C8161.9 cells were injected into mice (n = 10).

Melanoma Tissue Arrays and Immunostaining

The tissue microarrays utilized were previously created using core diameters of 1.0 mm taken from the paraffin blocks of patients (n = 311) (20,21) following a protocol approved by the institutional review boards at University of California San Francisco (UCSF) and California Pacific Medical Center (CPMC). Informed consent was obtained from each patient. Slides were prepared from formalin-fixed tissue microarrays and stained with anti-human BPTF antibody at a 1:100 dilution (Bethyl Laboratories, Montgomery, TX). Microwave antigen retrieval was conducted in 10 mM citrate buffer, pH 6.0. Endogenous peroxidase was blocked with 3% H2O2, and additional blocking was performed with normal rabbit serum. The primary antibody was diluted in 1.0% BSA in PBS and applied overnight at 4°C. Antibody staining was observed by using biotin-labeled anti-goat IgG and avidin-biotin (Vector Laboratories, Burlingame, CA), followed by diaminobenzidine. Sections were counterstained with hematoxylin.

Statistical Analysis

All quantified data represent an average of at least triplicate samples or as indicated. Error bars represent standard deviation. Statistical significance was determined by the Student’s t test, the Mantel-Cox log rank test, or using multivariable Cox regression. We examined graphic plots of the log cumulative hazard function for BPTF expression and found no violations of the proportional hazard assumption. All P values presented are two-tailed, and those under .05 were considered statistically significant.

Additional methods are provided in Supplementary Methods (available online).

Results

The role of Bptf was initially assessed using shRNA targeting in B16-F10 murine melanoma. Bptf expression was suppressed by two different shRNAs as measured by quantitative real-time polymerase chain reaction (qRT-PCR) and western blot analysis (Supplementary Figure 1, A-B, available online). Bptf silencing resulted in suppression of tumor cell proliferation (Supplementary Figure 1C, available online) and invasion into matrigel (Supplementary Figure 1D, available online). Subcutaneous injection of Bptf shRNA-expressing cells statistically significantly suppressed tumor cell growth by 67.1% in BPTF shRNA 2-expressing cells (P = .001) (Supplementary Figure 1E, available online). Intravenous inoculation of Bptf shRNA-expressing cells in C57Bl/6 mice showed a statistically significant reduction in metastatic tumor burden (by 83.8%) in BPTF shRNA 2-expressing cells (P = .001) (Supplementary Figure 1F, available online).
We used cDNA microarray analysis to identify the global patterns of gene expression following Bptf silencing. Statistical significance analysis of microarrays (22) comparing B16-F10 clones stably expressing Bptf shRNA 2 with the control vector identified 27 downregulated and 1008 upregulated genes (GEO accession number GSE64152). Of the downregulated genes, Bcl-xl and Cnd2 were selected for further characterization, given their known role in tumor progression. The differential expression of these genes (as well as Bcl-2) in B16-F10 melanoma was confirmed at the mRNA and protein levels (Supplementary Figure 1, G-H, available online).

Modulation of Human BPTF Expression

We then determined the consequences of regulating human BPTF expression. Overexpression of a distinct shRNA targeting human BPTF (shRNA 3) suppressed BPTF expression (by 78.2%) (Figure 1A) in BRAF-V600E-mutant, 1205-Lu melanoma cells, which exhibit elevated BPTF copy number (mean ± SD of 3.2 ± 0.8 vs 2.1 ± 0.5 for chromosome 17) by fluorescence in situ hybridization (FISH) analysis (Figure 1B). BPTF silencing led to G1/G0 cell cycle arrest and reduced S-phase (mean of control shRNA = 16.8%, 95% confidence interval [CI] = 15.2% to 18.4%; vs mean of BPTF shRNA = 11.2%, 95% CI = 9.76% to 12.6%; P = .01) (Figure 1C), decreased proliferative capacity of melanoma cells (by 65.5%) (Figure 1D), and increased apoptosis (13.3-fold, mean of control shRNA = 0.9%, 95% CI = 0.8% to 1.0%; vs mean of BPTF shRNA = 12.0%, 95% CI = 11.5% to 12.5%; P = .001) (Figure 1E). BPTF inhibition also led to a statistically significant decrease in 1205-Lu invasiveness (Supplementary Figure 2A, available online). Subcutaneous growth of 1205-Lu melanoma cells in nude mice was statistically significantly suppressed by 52.5% following BPTF shRNA expression (P = .02) (Figure 1F). Suppression of BPTF expression resulted in 66.4% reduction in the metastatic tumor burden in the lungs of nude mice upon intravenous 1205-Lu cell inoculation (Figure 1G). The suppressive effects of BPTF targeting on melanoma cell proliferation were confirmed using another shRNA (shRNA 4) targeting a different site within BPTF mRNA (Supplementary Figure 2B, available online). These antitumor effects were confirmed following shRNA-mediated BPTF suppression in C8161.9 human melanoma cells (Figure 2, A-E; Supplementary Figure 2C, available online), which also harbor elevated BPTF copy number (mean ± SD of 4.6 ± 1.5 vs 3.5 ± 1.2 for chromosome 17). These results confirm the protumorigenic role of BPTF in melanoma.

Signaling Cascade Activated by BPTF and Sensitivity to BRAF Inhibitors

We then examined the signaling pathways activated by BPTF in its regulation of melanoma proliferation and apoptosis. Initially, we confirmed the downregulation of BCL2, BCL-XL, and CCND2 following BPTF shRNA expression in 1205-Lu and C8161.9 human melanoma cells at both the RNA and protein levels (Figure 3, A-D). Conversely, BPTF overexpression in 1205-Lu cells resulted in statistically significantly increased (2.2-fold, P = .001) proliferative capacity (Figure 3E) and was accompanied by overexpression of BCL-XL, BCL2, and CCND2 (Figure 3, F-G).

We next examined the effects of BPTF silencing on the MAPK pathway, given the regulation of BCL2 and BCL-XL by ERK (23). BPTF suppression was accompanied by a reduction in total ERK levels in both 1205-Lu (by 58.4%) and C8161.9 (by 45.6%) melanoma cells, resulting in even more profound suppression of pERK (by 78.2% and 62.4%, respectively) (Figure 4, A-B). In addition, 1205-Lu and C8161.9 cells expressing anti-BPTF shRNA expressed lower levels of ERK1 and ERK2 RNA by qRT-PCR analysis (Supplementary Figure 2, E and F, available online). Next, we assessed whether BPTF expression directly activated ERK expression. FAC1, the truncated form of BPTF, exhibits sequence-specific DNA-binding activity (15), and analysis of the ERK promoter revealed a consensus BPTF-binding sequence. Cotransfection of either 1205-Lu or C8161.9 melanoma cells with a plasmid encoding the ERK promoter upstream of luciferase cDNA together with a plasmid encoding BPTF cDNA revealed 15-50-fold increased luciferase activity following BPTF overexpression, demonstrating transcriptional activation of ERK by BPTF in both melanoma cell lines (Figure 4, C and D). BPTF overexpression in 1205-Lu cells also resulted in increased expression of ERK1/2 mRNA and protein, including pERK (Figure 3, F and G). BPTF knockdown with shRNA 4 in 1205-Lu cells also suppressed expression of ERK1/2, pERK1/2, BCL-2, BCL-XL, and CCND2 (Supplementary Figure 2D, available online). Finally, overexpression of either ERK1/2 or BCL-XL cDNA in 1205-Lu cells expressing BPTF shRNA statistically significantly reversed anti-BPTF-mediated suppression of tumor cell proliferation (Figure 4E). These results indicate that the proliferative function of BPTF is mediated, at least in part, by ERK1/2 and BCL-XL.

ERK1/2, a downstream target of BRAF within the MAPK pathway, is suppressed following treatment with selective BRAF inhibitors (24,25) and can be reactivated upon resistance to these agents. Given the regulation of pERK1/2 by BPTF, we assessed whether BPTF expression levels modulated sensitivity to selective BRAF inhibitors. BPTF shRNA-expressing 1205-Lu cells were 2.8-fold more sensitive to vemurafenib treatment (Figure 5A) and 2.9-fold more sensitive to dabrafenib treatment (Figure 5B) than control shRNA-expressing cells. Conversely, BPTF overexpression in 1205-Lu cells statistically significantly reduced their sensitivity to vemurafenib or dabrafenib treatment (2.5-3.5-fold, respectively) (Figure 5, C and D). The regulation of sensitivity to anti-BRAF targeted agents by BPTF was confirmed in BRAF-mutant LOX and A375 human melanoma cells (Supplementary Figure 2, G-I, available online).

BPTF as a Molecular Marker of Melanoma Progression

We then performed a detailed assessment of BPTF’s biomarker role in melanoma. The human BPTF gene is located on 17q24.3, a locus which is amplified in various malignancies (10,12). We assessed BPTF copy number using FISH on 81 benign nevi and 77 primary melanomas. All nevi had two copies of BPTF, whereas 63.6% (49/77) of melanomas had more than two copies, and 36.4% (28/77) had more than three copies (Supplementary Table 1, available online). These findings indicate that BPTF copy number is elevated in a substantial proportion of melanomas, further supporting its pro-oncogenic role.

Next, we explored the prognostic role of BPTF in melanoma. Immunohistochemical analysis of BPTF expression in 311 primary melanomas (Supplementary Figure 3, A and B, available online) demonstrated a statistically significant...
Figure 1. Effects of suppression of bromodomain PHD finger transcription factor (BPTF) expression on 1205-Lu melanoma cells. A) Expression of BPTF mRNA in 1205-Lu cells following BPTF suppression. B) Fluorescence in situ hybridization analysis showing probe for BPTF (red) and probe for chromosome 17 (green). C) Cell cycle analysis of 1205-Lu cells following BPTF suppression. D) Colony formation assay following BPTF suppression in 1205-Lu cells. E) Apoptotic activity in 1205-Lu cells following BPTF knockdown. F) In vivo tumor cell growth (n = 10 mice per group) following BPTF knockdown (mean tumor volume of control shRNA on day 32 = 1770.3 mm³, 95% confidence interval [CI] = 257.4 to 3035.5; vs mean of BPTF shRNA 3 on day 32 = 841.6 mm³, 95% CI = 332.1 to 1527.1; P = .02). G) Metastatic lung tumor count following BPTF knockdown (n = 12 mice per group). In panels (A), (C), (D), (E), and (G), data presented reflect mean ± SD. Statistical significance was calculated by the two-tailed Student’s t test. *P < .05. In panel (B), scale bar reflects 20 μm. BPTF = bromodomain PHD finger transcription factor.
association between BPTF overexpression and both reduced distant metastasis-free survival (DMFS, \( P = .03 \), log-rank test) (Supplementary Figure 3C, available online) and disease-specific survival (DSS, \( P = .008 \), log-rank test) (Supplementary Figure 3D, available online). By multivariable Cox regression analysis, BPTF overexpression independently predicted DMFS and DSS (Supplementary Table 2, available online). Thus, BPTF expression is predictive of the development of distant metastasis and reduced survival in melanoma patients, and is an independent predictor of survival.

In addition, we analyzed BPTF levels in individual tumor samples from eight metastatic melanoma patients prior to initiation of and following progression on vemurafenib or dabrafenib. In a patient treated with vemurafenib (case M4331), immunohistochemical and FISH analysis indicated homogeneous BPTF staining (Figure 6A-C). In stark contrast, in the progressing specimen, BPTF immunostaining was heterogeneous, with clones of cells displaying absent BPTF expression (suggestive of cells responding to treatment) and another clone of cells with high BPTF expression (suggestive of resistant cells) (Figure 6D and E). Immunohistochemical analysis using antibodies targeting the melanocyte antigens MART1, HMB45, and tyrosinase revealed positive staining in both of these regions (Figure 6F), confirming their melanocytic lineage. TUNEL staining identified the low BPTF-expressing cells as having undergone apoptosis, while the high BPTF-expressing cells displayed minimal TUNEL staining (Figure 6G). FISH analysis also revealed profound clonal heterogeneity at the DNA level (Supplementary Table 3, available online). Specifically, the region containing highly apoptotic, low BPTF-expressing cells had no FISH signals (Figure 6H), whereas the region with high BPTF immunostaining harbored cells with high BPTF copy number (without evidence of amplification) (Figure 6I). FISH analysis of the transition zone between these regions showed a near-diploid copy number (Figure 6J). Similar findings are shown in a patient treated with dabrafenib (case M317) (Supplementary Figure 4, available online). Overall, in six out of eight patients progressing on anti-BRAF therapy, tumors were characterized by marked heterogeneity in BPTF expression, whereas all tumors harvested prior to therapy exhibited homogeneous BPTF staining (Supplementary Table 4, available online). These results indicate that increased levels of BPTF can mediate resistance to BRAF inhibitors and can be selected for during acquired resistance to these agents. In addition, tumors progressing following BRAF inhibition are characterized by marked heterogeneity in BPTF copy number and expression, suggesting a differential response to treatment within the tumor.

**Discussion**

Chromatin-remodeling factors have been recognized to play an increasingly important role in tumorigenesis (26), given the demonstration of mutations in chromatin regulators in various human cancers (27–29). In addition, the 17q24 locus has been reported to be amplified in breast cancer (10), and increased 17q24 copy number has been observed in other solid tumors (11,12,30,31). However, which genes within this locus are responsible for its protumorigenic properties

![Figure 2](https://example.com/figure2.png)

**Figure 2.** Effects of bromodomain PHD finger transcription factor (BPTF) suppression on C8161.9 human melanoma cells. A) BPTF mRNA expression following BPTF suppression in C8161.9 melanoma cells. B) Fluorescent in situ hybridization analysis showing probe for BPTF (red) and probe for chromosome 17 (green). C) Proliferative activity following BPTF knockdown as determined by a colony formation assay. D) Apoptotic activity in C8161.9 melanoma cells following BPTF suppression. E) In vivo tumor growth following BPTF shRNA expression. In panels (A), (C), and (D), data presented reflect mean ± SD. In panel (E), \( n = 10 \) for each group (mean tumor volume of control shRNA at day 28 = 1538.5, 95% confidence interval [CI] = 770.1 to 2567.9; vs mean of BPTF shRNA 3 at day 28 = 457.5, 95% CI = 200.9 to 744.5). Statistical significance was calculated by the two-tailed Student’s t test. *\( P < .05 \) in panel (B), scale bar reflects 20 μm. BPTF = bromodomain PHD finger transcription factor.
has remained unclear. BPTF (whose gene resides on 17q24) has been implicated in embryonic development (4), thymocyte maturation (32), and chromatin remodeling (6). However, little is known about the functional role played by BPTF in tumorigenesis.

In this study, we document the functional and biological significance of BPTF in melanoma progression. BPTF silencing suppressed melanoma cell proliferation, while BPTF overexpression promoted in vitro tumor cell growth. In vivo studies confirmed the potent role played by BPTF in promoting tumor progression, as evidenced by decreased tumor cell growth and/or metastatic potential using different shRNAs targeting both murine and human BPTF. Integrated analyses provided mechanistic insight into BPTF function, identifying BCL2, BCL-XL, and CCND2 as key markers regulated by BPTF in promoting tumor cell proliferation and cell cycle progression, and in inhibiting apoptosis (33–35).

Biomarker analyses revealed elevated BPTF copy number in 36.4% of melanoma. In contrast, increased BPTF copy number was absent in benign nevi, suggesting the potential utility of using BPTF as a diagnostic marker to distinguish melanoma from nevi. Increased BPTF copy number provides a potential mechanism for BPTF activation in melanoma, as suggested by its overexpression using cDNA microarray analysis. BPTF overexpression was an independent predictor of reduced DMFS and DSS in human melanoma patients. Thus, BPTF is both a predictor and a promoter of distant metastasis, the lethal event in melanoma progression.
Suppression of BPTF expression produced pronounced anti-tumor effects in melanoma cells and resulted in substantially reduced levels of ERK, a downstream marker of MAPK pathway activation. Overexpression of cDNAs encoding either BCL-XL or ERK partially reversed the suppression of melanoma cell growth produced by BPTF shRNA expression. Importantly, BPTF overexpression directly activated ERK at the transcriptional level, identifying it as a potent activator of the MAPK pathway, a key mediator of tumor cell survival. In accord with this observation, modulation of BPTF expression markedly modulated the sensitivity of mutant-BRAF melanoma cells to selective BRAF inhibitors.

Treatment of metastatic melanoma patients harboring mutant BRAF with selective BRAF inhibitors has been shown to
conferring an overall survival advantage (36,37). However, complete responses are rare and acquired resistance to these agents develops in the majority of treated patients, resulting in the cessation of therapy. Several mechanisms of acquired resistance have been described, including reactivation of the MAPK pathway or activation of the PI3K pathway (38,39). Our studies provide new evidence for the functional role of this chromatin-remodeling factor in promoting acquired resistance to BRAF-targeted therapies. Specifically, the increased BPTF copy number observed in progressing tumors suggests that BPTF activation may be selected for during the acquisition of resistance to BRAF-targeted therapy.

Surprisingly, analysis of specimens following progression with selective BRAF inhibitor therapy identified substantial intratumor molecular heterogeneity upon the development of acquired resistance. One subclone of tumor cells, devoid of BPTF staining and highly apoptotic, appeared to have maintained response to therapy, while the other subclone, with prominent BPTF staining and low apoptotic rates, represented the predominant resistant cell population. In addition, a second layer of molecular heterogeneity was observed, as the surviving, high BPTF-expressing cells themselves were characterized by distinct subclones with differing BPTF copy numbers. Thus, metastatic tumors resistant to BRAF-targeted therapy do not appear homogeneously resistant at the cellular and molecular levels. Rather, they are composed of cell subpopulations that have responded to the targeted intervention. These observations extend the recent demonstration of intratumoral molecular heterogeneity in a small number of renal cell carcinomas (40) to the realm of resistance to targeted therapy.

Recently, BPTF was among a group of chromatin-remodeling factors mutated in liver cancers by whole-genome sequencing (27). Transient siRNA targeting of BPTF was accompanied by increased proliferation in two of five liver cancer cell lines, suggesting a loss-of-function role for these mutations. However, no additional functional studies were provided to explore the consequences of BPTF targeting. BPTF mutations have been demonstrated in a small number of skin cancers, including melanoma (41). However, sequencing of the mutated loci observed in skin cancers in our samples did not identify any mutations in 35 melanomas examined, including in specimens obtained from patients progressing on anti-BRAF agents.

By contrast, our studies assign novel, protumorigenic roles to BPTF in melanoma, driven in part by increased BPTF copy number. Given the development of bromodomain inhibitors to target the BET protein family (42), our results suggest BPTF targeting as a novel approach to overcome resistance to BRAF inhibitors, including a possible combinatorial treatment for BRAF-mutant melanoma. However, specific inhibitors targeting
the BPTF bromodomain would need to be developed, and may be expected to affect the developing nervous system or thymocyte function. Finally, given the presence of apoptotic subclones of tumor cells within drug-resistant tumors, these results suggest that treatment with selective BRAF inhibitors may potentially be continued following the development of resistance, as part of a combinatorial strategy.

Our study is not without limitations. The 1205-Lu cell line contains both mouse and human chromosomes (43), even though it does harbor both a human BRAF-V600E mutation and an elevated human BPTF copy number. However, the regulation of sensitivity to BRAF inhibitors by BPTF was also demonstrated in LOX and A375 cells, providing important additional support for the conclusions drawn. In addition, it is possible that regulation of ERK by BPTF occurs indirectly. Finally, the diagnostic and prognostic roles for BPTF need to be confirmed in additional cancers, and the demonstration of intratumoral heterogeneity following targeted therapy deserves examination in a larger patient cohort.

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