

AWARD NUMBER: W81XWH-13-1-0159

TITLE: Treatment of Endocrine-Resistant Breast Cancer with a Small Molecule c-Myc Inhibitor

PRINCIPAL INVESTIGATOR: Qin Feng

CONTRACTING ORGANIZATION: Baylor College of Medicine
Houston, TX 77030

REPORT DATE: June 2015

TYPE OF REPORT: Annual Report

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. **PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.**

1. REPORT DATE June 2015			2. REPORT TYPE Annual Report		3. DATES COVERED 1 Jun 2014 - 31 May 2015	
4. TITLE AND SUBTITLE Treatment of endocrine-resistant breast cancer with a small molecule c-myc inhibitor					5a. CONTRACT NUMBER	
					5b. GRANT NUMBER W81XWH-13-1-0159	
					5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Qin Feng E-Mail: qfeng@bcm.edu					5d. PROJECT NUMBER	
					5e. TASK NUMBER	
					5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Baylor College of Medicine One Baylor Plaza Houston, TX77030					8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012					10. SPONSOR/MONITOR'S ACRONYM(S)	
					11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited						
13. SUPPLEMENTARY NOTES						
14. ABSTRACT Breast cancer is the most common cancer in women. Tamoxifen has been a front-line treatment for estrogen receptor alpha (ER α)-positive breast tumors in premenopausal women. However resistance to tamoxifen occurs in many patients. ER α still plays a critical role in the growth of breast cancer cells with acquired tamoxifen resistance, suggesting that ER α remains a valid target for treatment of tamoxifen-resistant breast cancer. In an effort to identify novel regulators of ER α signaling, through a small-scale siRNA screen against histone methyl modifiers, we found WHSC1, a histone H3K36 methyltransferase, as a positive regulator for ER α signaling in breast cancer cells. We demonstrated that WHSC1 is recruited to the ER α gene by interacting with the BET protein BRD3/4, and facilitates ER α gene expression. The small-molecule BET protein inhibitor JQ1 potently suppressed the classic ER α signaling pathway and the growth of tamoxifen-resistant breast cancer cells in culture. Through the second year of funding, we have made the following discovery: 1. We have successfully dissected the molecular mechanisms of how WHSC1 complexes with BET proteins, and subsequently regulates the ER α gene expression at the chromatin level; 2. We have shown that JQ1 has potent anti-tumor activity against tamoxifen-resistant and estrogen-deprivation-resistant breast cancer cells in multiple endocrine-resistant models; 3. We have demonstrated that <i>in vivo</i> , JQ1 has anti-cancer activity against tamoxifen-resistant tumor; most importantly, we have demonstrated that JQ1 potently inhibits growth of tamoxifen-resistant tumor <i>in vivo</i> when combined with fulvestrant therapy.						
15. SUBJECT TERMS Endocrine, tamoxifen, bromodomain, resistance						
16. SECURITY CLASSIFICATION OF:				17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON USAMRMC
a. REPORT	b. ABSTRACT	c. THIS PAGE	19b. TELEPHONE NUMBER (include area code)			
Unclassified	Unclassified	Unclassified	Unclassified	12		

Table of Contents

	<u>Page</u>
1. Introduction.....	4
2. Keywords.....	4
3. Overall Project Summary.....	4-6
4. Key Research Accomplishments.....	6
5. Conclusion.....	6-7
6. Publications, Abstracts, and Presentations.....	7
7. Inventions, Patents and Licenses.....	7
8. Reportable Outcomes.....	7
9. Other Achievements.....	8
10. References.....	8
11. Appendices.....	8-12

TREATMENT OF ENDOCRINE-RESISTANT BREAST CANCER WITH A SMALL MOLECULE C-MYC INHIBITOR

Introduction

Breast cancer is the most common cancer in women. Tamoxifen has been a front-line treatment for estrogen receptor alpha (ER α)-positive breast tumors in premenopausal women. However resistance to tamoxifen occurs in many patients. ER α still plays a critical role in the growth of breast cancer cells with acquired tamoxifen resistance, suggesting that ER α remains a valid target for treatment of tamoxifen-resistant breast cancer. In an effort to identify novel regulators of ER α signaling, through a small-scale siRNA screen against histone methyl modifiers, we found WHSC1, a histone H3K36 methyltransferase, as a positive regulator for ER α signaling in breast cancer cells. We demonstrated that WHSC1 is recruited to the ER α gene by interacting with the BET protein BRD3/4, and facilitates ER α gene expression. The small-molecule BET protein inhibitor JQ1 potently suppressed the classic ER α signaling pathway and the growth of tamoxifen-resistant breast cancer cells in culture. It was reported that JQ1 functions mainly by down-regulating MYC transcription [1, 2]. My results suggest that JQ1 might be a useful drug in treatment of Tamoxifen resistant breast cancer by shutting down the expression of both ER α and MYC at the transcriptional level. In this grant, I proposed to (1) Determine the molecular mechanisms of JQ1 effect on Tamoxifen-resistant MCF7L breast cancer cells by microarray analysis; (2) Determine the therapeutic effect of JQ1 on a panel of well-characterized endocrine resistant preclinical models; (3) Study JQ1 in vivo anti-tumor function using the Tamoxifen-resistant breast cancer xenograft model in nude mouse.

Keywords

Breast cancer, Endocrine-resistant, Tamoxifen, MYC, JQ1

Overall Project Summary

During the second year, we have examined the therapeutic effect of JQ1 on a panel of endocrine resistant breast cancer cell line models, and performed in vivo experiments to determine the effect of JQ1 on tamoxifen-resistant xenograft model after resistance is established. Specifically, the following are the results we obtained (**figures and legends are included in the Appendices section**):

1. Further dissecting the molecular mechanisms of how JQ1 affect ER α gene expression.

1a. BRD4 interacts with WHSC1 through its N-terminal 470 amino acids.

Previously we identified WHSC1 as a regulator for ER α gene expression, and BRD3 and BRD4 have been identified as interacting proteins of WHSC1. BRD3 and BRD4 belong to the BET (bromodomain and extraterminal domain) family of proteins. In humans, there are four BET proteins including BRD2, BRD3, BRD4, and BRDT [3]. BET family members contain two bromodomain and one extraterminal domain. The bromodomain specifically recognizes acetylated lysine residues on the histone tails. BRD3 and BRD4 are implicated in the transcription elongation process by association with the PAF1 complex and the pTEFb

complex, respectively [4]. BRD4 also regulates the expression of G1 cell cycle genes. We next determined the domain responsible for the interaction between WHSC1 and BRD3/4 by deletion mapping. Shown in Fig. 1a and 1b, BRD4 interacts with WHSC1 through its NH2-terminal 470 amino acids, which contains two bromodomains. Interestingly, treatment of cells with JQ1 failed to disrupt the interaction between WHSC1 and BRD4, suggesting BRD4 can bind to WHSC1 and acetylated lysine simultaneously (Fig. 1C).

1b. Loss of BRD3/4 abolished the recruitment of WHSC1 to ER α gene.

To further test the hypothesis that WHSC1 is recruited to the ER α gene promoter through BRD3 and BRD4, these two genes were knocked down in MCF7 cells, and the recruitment of WHSC1 to the ER α gene and the level of histone H3 K36 methylation were determined by ChIP-qPCR assay. As shown in Fig. 2, knockdown of BRD3/4 dramatically reduced the recruitment of WHSC1 to the ER α gene. Consistent with the fact that WHSC1 is a methyltransferase for histone H3K36, the levels of H3K36me2 and H3K36me3 were both significantly reduced, suggesting that BRD3/4 functions as a scaffold to recruit WHSC1, which promotes the transcription elongation of the ER α gene.

2. JQ1 has anti-tumor activity in multiple tamoxifen-resistant (Tam-R) and estrogen-deprivation-resistant (EDR) breast cancer cells.

Previously we have investigated if the BRD3/4 inhibitor JQ1 inhibits growth of breast cancer cells. We treated parental MCF7 cells and a tamoxifen-resistant derivative with different doses of JQ1 or vehicle control. JQ1 has shown potent growth inhibition in Tam-R MCF7 cells, whereas it only moderately inhibited the growth of parental MCF7 cells at a concentration of 0.2 μ M. Here we further tested three more ER+ breast cancer cell lines including T47D, MCF7RN, and ZR75-1 cells. We found that JQ1 inhibited the growth of all of these breast cancer cell lines, with higher efficacy towards the tamoxifen resistant cells. Interestingly, JQ1 also inhibited four estrogen-deprivation-resistant (EDR; mimics aromatase inhibitor resistance) lines (Fig. 3a). Similar to MCF7 cells, JQ1 downregulated ER α mRNA levels in MCF7RN and ZR75-1 cells. We also measured the mRNA level of ER α dominant negative splicing variant ER Δ 7, the major alternatively spliced form in most human breast tumors and cancer cell lines [5]. Similarly to wild-type ER α , the mRNA level of ER Δ 7 is also downregulated by JQ1 treatment (Fig. 3b and 3c).

3. JQ1 has *in vivo* anticancer activity against tamoxifen-resistant breast cancer

Next we sought to determine the *in vivo* anti-tumor activity of JQ1. Ovariectomized nude mice were transplanted subcutaneously with Tam-R MCF7 tumors, and were randomized into two groups when tumor volumes reached 200 mm³. JQ1 or control vehicle was given to these mice by intraperitoneal injection daily. A pilot experiment was performed to treat the mice for 7 days to test if JQ1 could downregulate ER α expression *in vivo*. As shown in Fig. 4a, the ER α mRNA level was indeed reduced about 30% in JQ1 treated tumors. MYC mRNA level was not significantly reduced, although there was a trend. Immunochemical staining confirmed a decreased level of ER α protein in JQ1-treated tumors, and a reduced proliferation rate was observed with Ki67 and histone H3 phospho-Ser10 staining (Fig. 4b).

This result demonstrates that JQ1 has *in vivo* anticancer activity against tamoxifen-resistant breast cancer.

4. JQ1 potently inhibits growth of tamoxifen-resistant tumor *in vivo* when combined with fulvestrant therapy

In our pilot experiment, JQ1 exhibits mild *in vivo* anticancer activity against tamoxifen-resistant breast tumor when JQ1 was used alone. Gene expression and histochemical staining indicates that ER α mRNA level has mild reduction upon JQ1 treatment, but far from the potent inhibition effect as what we observed in cultured cells (Fig. 4). In order to further downregulate ER α and achieve an optimal drug response *in vivo*, we tested a combination of JQ1 and fulvestrant/ICI 182,780, an ER α protein degrader, in Tam-R MCF7 xenograft tumors. While single treatment of JQ1 or fulvestrant moderately inhibited tumor growth, the combination of JQ1 and fulvestrant showed a synergistic anti-tumor activity in the Tam-R tumors. In the vehicle-treated group, the volumes of all the tumors quickly tripled within 17 days, while in the group that received combination therapy, none of the tumors tripled their size after 40 days of treatment, and only about half of the tumors tripled after a prolonged treatment (90 days) (Fig. 5a). Time-to-tumor tripling from the four groups of treatment was compared using the generalized Wilcoxon test as shown in Fig. 6. Although JQ1-treated animals lose weight initially and then regain it, generally there is no difference among the four groups in the baseline weights, indicating that the JQ1 is well tolerated by mice (Fig. 5b). By Western blot analysis, the protein levels of ER α were dramatically downregulated in the tumors that received combination therapy of fulvestrant and JQ1, despite that single treatment of each drug only partially decreased ER α protein level (Fig. 5c). Immunochemical staining for proliferation markers Ki67 and histone H3 phospho-Ser10 confirmed that the combination therapy potently inhibited the tumor cell proliferation (Fig. 5d).

Key Research Accomplishments

Through the second year of funding we have made the following research accomplishments:

- We have successfully dissected the molecular mechanisms of how the histone methyltransferase WHSC1 complexes with acetylated histone binding protein BRD3/4, and how this complex regulate ER α gene expression at the chromatin level;
- We have shown that JQ1 has potent anti-tumor activity against tamoxifen-resistant (Tam-R) and estrogen-deprivation-resistant (EDR) breast cancer cells in multiple resistant models;
- We have demonstrated that *in vivo*, JQ1 has anti-cancer activity against tamoxifen-resistant tumor;
- Most importantly, we have demonstrated that JQ1 potently inhibits growth of tamoxifen-resistant tumor *in vivo* when combined with fulvestrant therapy.

Conclusion

The central idea of this study is to apply a novel epigenetic drug, JQ1, to treat endocrine-resistant breast cancer. Previously we have found that JQ1 inhibits the growth of cultured tamoxifen-resistant breast cancer cells by down-regulating ER α gene expression, and microarray analysis suggested that the cell

cycle is an important pathway being affected by JQ1, because cell cycle-related gene expression was significantly altered by JQ1 treatment. Consistently, JQ1 also exhibits anti-tumor effect in multiple ER-positive endocrine resistant models, including MCF7, MCF7RN, T47D, and ZR75-1 cell models. More importantly, JQ1 shows *in vivo* anti-cancer activity in suppressing the tamoxifen-resistant breast cancer growth in the xenograft mouse model. Using a combination of fulvestrant with JQ1 to more effectively down-regulate ER α and inhibit *in vitro* and *in vivo* tumor growth, our results provide a new potential approach for treating tamoxifen-resistant and ER α -dependent breast cancer.

Publications, Abstracts and Presentations

1. I have generated 4 publications during the second year of funding:

- Feng Q, Zhang Z, Shea MJ, Creighton CJ, Coarfa C, Hilsenbeck SG, Lanz R, He B, Wang L, Fu X, Nardone A, Song Y, Bradner J, Mitsiades N, Mitsiades CS, Osborne CK, Schiff R, O'Malley BW. 2014. An epigenomic approach to therapy for tamoxifen-resistant breast cancer. *Cell Res.* 24(7): 809-19. PMID: 24874954
- Feng Q, O'Malley BW. 2014. Nuclear Receptor Modulation – Role of coregulators in selective estrogen receptor modulator (SERM) actions. *Steroids.* 90: 39-43. PMID: 24945111
- He B, Lanz R, Fiskus W, Geng C, Yi P, Hartig SM, Rajapakshe K, Shou J, Wei L, Shah SS, Foley C, Chew SA, Eedunuri VK, Bedoya DJ, Feng Q, Frolov A, Weigel NL, Hilsenbeck SG, Rosen DG, Palzkill TG, Ittmann MM, Song Y, Coarfa C, O'Malley BW, Mitsiades N. 2014. GATA2 facilitates steroid receptor coactivator (SRC) recruitment to the androgen receptor (AR) complex. *Proc Natl Acad Sci U S A.* 111(51):18261-6. PMID: 25489091
- Yi P, Wang Z, Feng Q, Pintilie GD, Foulds CE, Lanz RB, Ludtke SJ, Schmid MF, Chiu W, O'Malley BW. 2015. The structure of a biologically active estrogen receptor-coactivator complex on DNA. *Mol Cell.* 57(6): 1047-58. PMID: 25728767

2. I have attended the 2014 Cold Spring Harbor Asia conference (Epigenetics, Chromatin & Transcription), and gave an oral presentation “An epigenomic approach to therapy for tamoxifen-resistant breast cancer”.

Inventions, Patents and Licenses

Nothing to report

Reportable Outcomes

Our work has demonstrated that the small-molecule BET protein inhibitor JQ1 potently suppressed the classic ER α signaling pathway and the growth of tamoxifen-resistant breast cancer cells in culture. Using a tamoxifen-resistant breast cancer xenograft mouse model, we are the first to show the *in vivo* anti-breast cancer activity by JQ1 and a strong long-lasting effect of combination therapy with JQ1 and the ER degrader fulvestrant. Thus we provide evidence that the epigenomic proteins BRD3/4 and WHSC1 are essential regulators of estrogen receptor signaling and are novel therapeutic targets for treatment of tamoxifen-resistant breast cancer.

Other achievements

1. Our work has established a novel therapeutic strategy to treat ER-positive breast cancer. Based on our study, it has been proposed that BET protein inhibitors might be used to attack recurrent ER+ breast cancers, particularly in breast cancers with ER fusion mutants, which cannot be targeted by traditional endocrine therapy (personal communication with Dr. Matthew Ellis, the director of Lester and Sue Smith Breast Center at Baylor College of Medicine).
2. Based on part of the work supported by this award, last year I have submitted a DoD BCRP Breakthrough Award application entitled: 'Targeting S-Adenosylhomocysteine Hydrolase for Treatment of Endocrine-Resistant Breast Cancer' and currently is on pending.
3. We have generated multiple endocrine-resistant breast cancer cell models and are available for scientific field upon request.

References

1. Mertz, J.A., et al., *Targeting MYC dependence in cancer by inhibiting BET bromodomains*. Proc Natl Acad Sci U S A, 2011. **108**(40): p. 16669-74.
2. Delmore, J.E., et al., *BET bromodomain inhibition as a therapeutic strategy to target c-Myc*. Cell, 2011. **146**(6): p. 904-17.
3. Wu, S.Y. and C.M. Chiang, *The double bromodomain-containing chromatin adaptor Brd4 and transcriptional regulation*. J Biol Chem, 2007. **282**(18): p. 13141-5.
4. Dawson, M.A., et al., *Inhibition of BET recruitment to chromatin as an effective treatment for MLL-fusion leukaemia*. Nature, 2011. **478**(7370): p. 529-33.
5. Garcia Pedrero, J.M., et al., *The naturally occurring variant of estrogen receptor (ER) ERDeltaE7 suppresses estrogen-dependent transcriptional activation by both wild-type ERalpha and ERbeta*. Endocrinology, 2003. **144**(7): p. 2967-76.

Appendices

Figures and legends (see following pages)

Supporting data:

Fig. 1

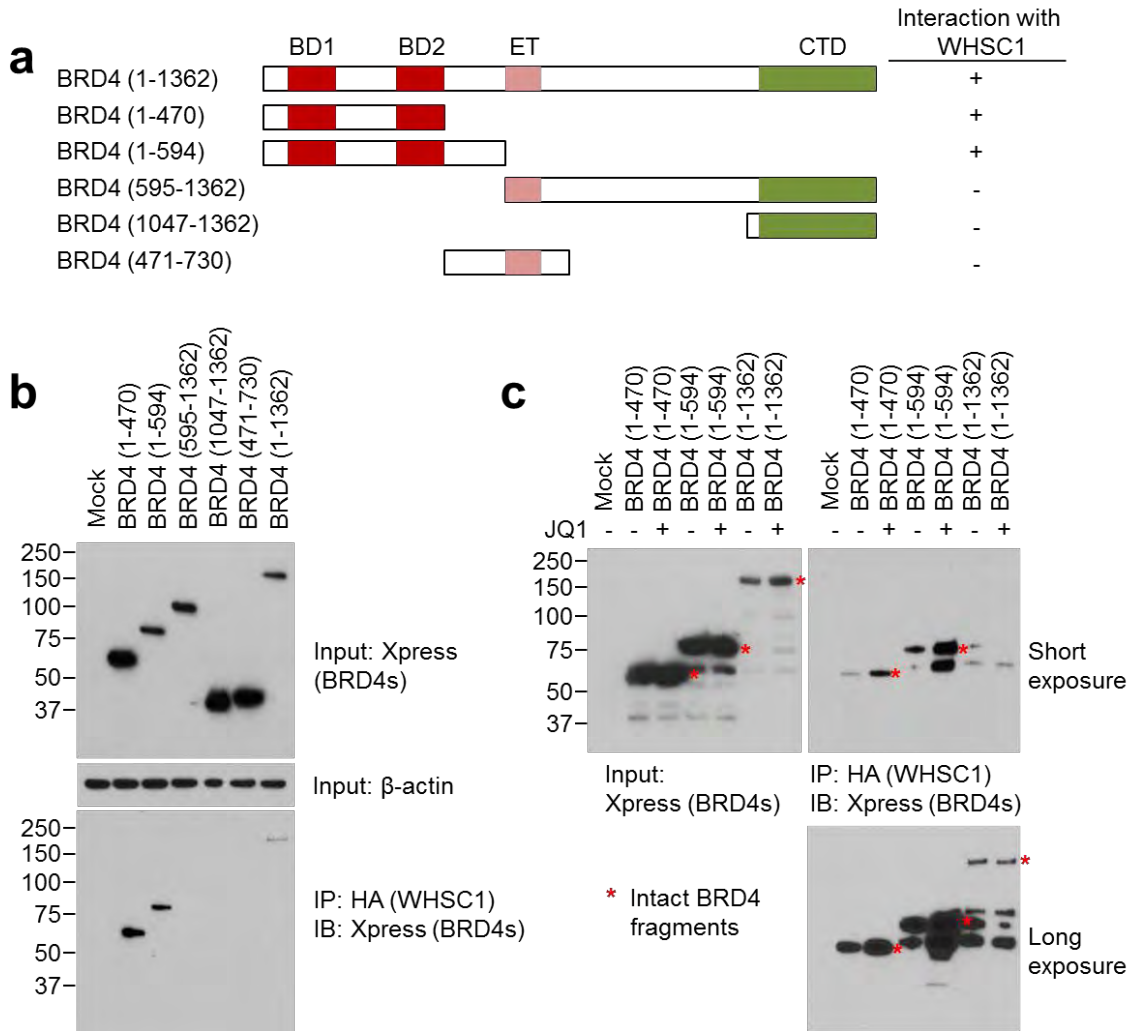


Fig. 1. Deletion mapping of the interacting region between BRD4 and WHSC1. (a) Diagram of BRD4 deletion mutants and the result of their interactions with WHSC1. BD, bromodomain; ET, extraterminal domain; CTD, C-terminal domain. (b) Interaction between WHSC1 and BRD4 deletion mutants by Co-IP experiment. 293T cells were transiently transfected with vectors expressing HA-tagged WHSC1 and Xpress-tagged BRD4 deletion mutants. IP antibody: anti-HA; blot antibody: anti-Xpress. (c) JQ1 does not interfere with the interaction between WHSC1 and BRD4. 0.5 μ M of JQ1 was added to cell culture medium 1 hour before cell harvest. Same concentration of JQ1 was also supplemented in cell lysates with JQ1 treatment during immunoprecipitation. A long exposure image is included to show the immunoprecipitated full-length BRD4 protein. The intact BRD4 fragments were marked by red asterisks.

Fig. 2

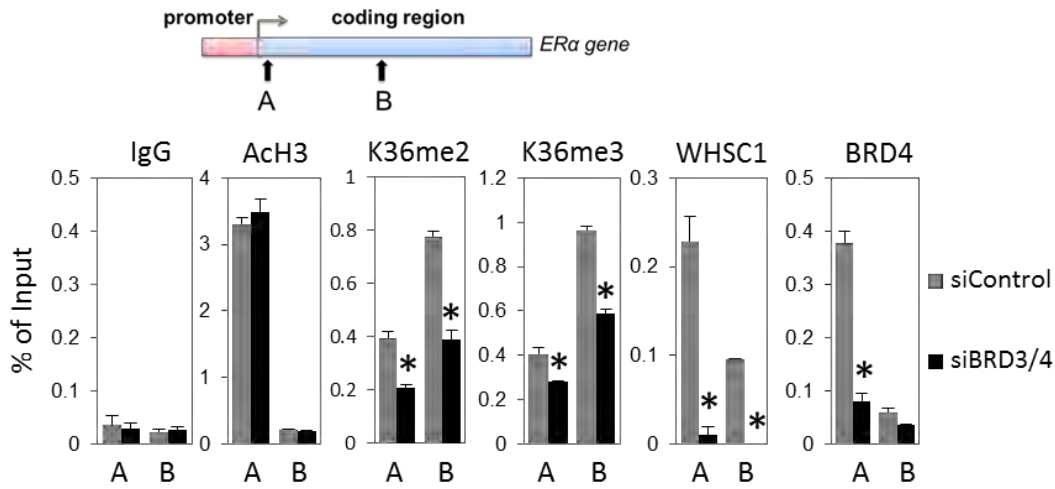


Fig. 2. Loss of BRD3/4 abolished the recruitment of WHSC1 to ERα gene. Chromatin-IP was performed in MCF7 cells treated with BRD3/4 siRNA or control siRNA for 2 days. Primer pair A locates next to promoter region and primer pair B locates in the gene body region of ERα. Each IP was duplicated and average values were shown. Error bars indicate s.e.m. *, p<0.05 by t-test.

Fig. 3

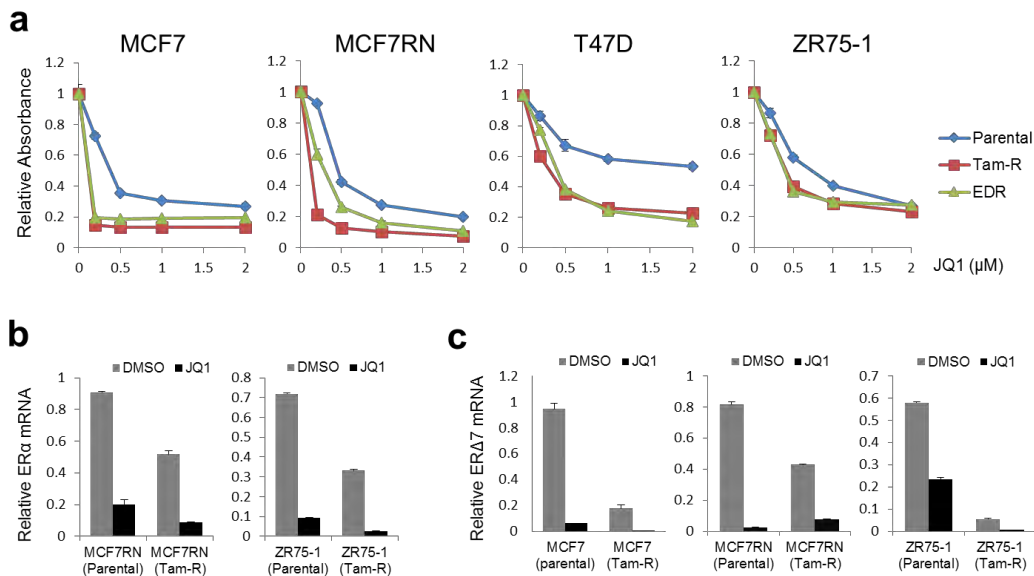


Fig. 3. (a) JQ1 inhibits the growth of multiple Tam-R and EDR (estrogen-deprivation-resistant) breast cancer cells. The anti-tumor effect of JQ1 was evaluated in several ER-positive, Tam-R, or EDR cell lines including MCF7, T47D, MCF7RN, and ZR75-1. Cell growth was determined after 5 days of JQ1 treatment. Error bars were shown as s.e.m. (b) JQ1 downregulates ERα gene expression in multiple ER-positive breast cancer cell lines. Here MCF7RN and ZR75-1 parental and Tam-R cells were tested. 0.5 μM of JQ1 was used to treat the cells 24 hours before harvest. (c) JQ1 also reduces expression of ERΔ7, a major ERα isoform, in multiple ER-positive cell lines. 0.5 μM of JQ1 was used to treat the cells 24 hours before harvest.

Fig. 4

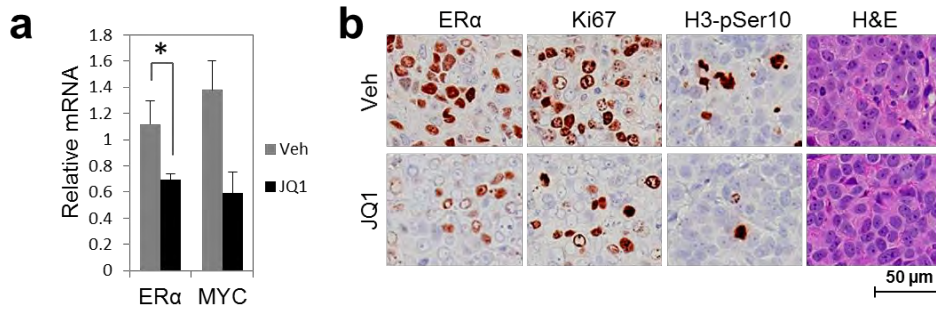


Fig. 4. (a) JQ1 downregulates ERα mRNA in JQ1-treated xenograft tumor tissue. JQ1 was administered by intraperitoneal injection at 50 mg/kg daily for 7 days. 6 mice per group. *, $p < 0.05$ by t-test. Error bars were shown as s.e.m. (b) Immunohistochemistry staining of ERα, Ki67 and histone H3 phospho-serine 10 in xenograft Tam-R tumor from Vehicle or JQ1 treated group.

Fig. 5

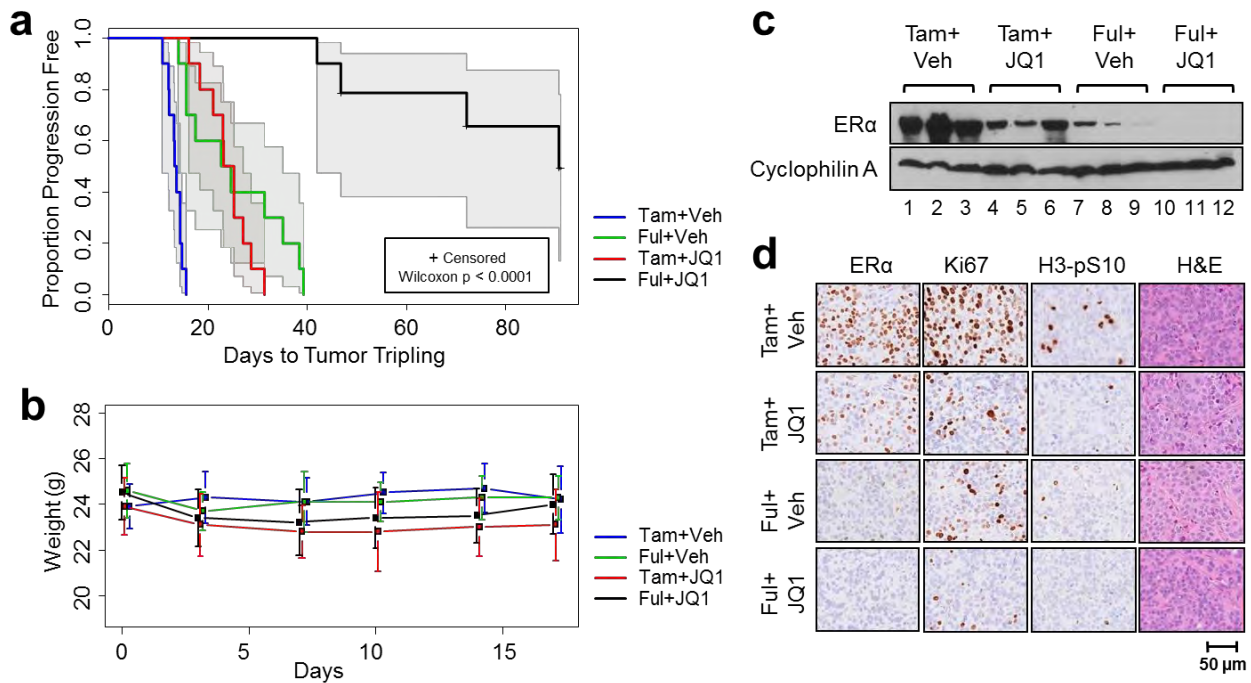


Fig. 5. A combination therapy of JQ1 and fulvestrant in Tam-R xenograft mouse model. (a) Ovariectomized mice bearing Tam-R established MCF7 tumors were randomized (on day 0) into 4 groups of treatment: Tam+Vehicle, Tam+JQ1, fulvestrant+Vehicle, and fulvestrant+JQ1, with 10 mice per group. JQ1 was administered daily at 50 mg/kg, while 5 mg fulvestrant was given by subcutaneous injection weekly. Tumors were harvested when they reached 1000 mm³ or three months after treatment. (b) Body weight measurement for xenograft experiment shown in (a). The error bars show means +/- s.e.m. (c) Tumors were harvested by the end of the treatment, and Western blot was performed using antibodies against ERα and cyclophilin A. (d) Immunohistochemical staining of ERα, Ki67 and histone H3 phospho-serine 10 in xenograft Tam-R tumors from four groups treated with Tam+Vehicle, Tam+JQ1, fulvestrant+Vehicle, or fulvestrant+JQ1.

Fig. 6

The *LIFETEST* Procedure

Stratum 1: Fulvestrant + JQ1

Quartile Estimates				
95% Confidence Interval				
Percent	Point Estimate	Transform	Lower	Upper
75		LOGLOG	90.7000	
50	90.7000	LOGLOG	42.0000	
25	72.0357	LOGLOG	42.0000	

Stratum 3: Tam + Vehicle

Quartile Estimates				
95% Confidence Interval				
Percent	Point Estimate	Transform	Lower	Upper
75	14.3865	LOGLOG	13.2714	15.5455
50	13.4754	LOGLOG	10.8636	14.3865
25	12.1205	LOGLOG	10.8636	13.6793

Test of Equality over Strata			
Test	Chi-Square	DF	Pr > Chi-Square
Wilcoxon	56.6203	3	<.0001

Stratum 2: Tam + JQ1

Quartile Estimates				
95% Confidence Interval				
Percent	Point Estimate	Transform	Lower	Upper
75	35.0191	LOGLOG	22.5904	39.2530
50	23.5931	LOGLOG	14.0000	35.0191
25	15.5904	LOGLOG	14.0000	24.5957

Stratum 4: Fulvestrant + Vehicle

Quartile Estimates				
95% Confidence Interval				
Percent	Point Estimate	Transform	Lower	Upper
75	27.1024	LOGLOG	23.0508	31.3444
50	24.1017	LOGLOG	16.1034	27.1024
25	21.0517	LOGLOG	16.1034	25.1527

Adjustment of Multiple Comparisons for the Wilcoxon Test				
Strata Comparison		Chi-Square	p-Values	
treatment	treatment		Raw	Sidak
Fulvestrant + JQ1	Tam + JQ1	7.5171	0.0061	0.0361
Fulvestrant + JQ1	Tam + Vehicle	45.7664	<.0001	<.0001
Fulvestrant + JQ1	Fulvestrant + Vehicle	6.8020	0.0091	0.0534
Tam + JQ1	Tam + Vehicle	10.8938	0.0010	0.0058
Tam + JQ1	Fulvestrant + Vehicle	0.0143	0.9049	1.000
Tam + Vehicle	Fulvestrant + Vehicle	11.7782	0.0006	0.0036

Fig. 6. Time to tumor tripling from four treatment groups (Tam+Vehicle, Tam+JQ1, fulvestrant+Vehicle, and fulvestrant+JQ1, with 10 mice per group) was compared using the generalized Wilcoxon test. Adjustments for multiple comparisons were also shown.