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14. ABSTRACT

Triple-negative breast cancer (TNBC) distinguishes from other forms of breast cancer in origination and progression. Likely originated from undifferentiated cancer stem cells, TNBC tumor cells possess many epithelial-mesenchymal transition (EMT) characteristics including invasion, resistance to apoptosis, and cancer stem cell-like traits that permit tumor dissemination and growth at distant sites. The Wnt pathways are important for EMT. We recently discovered that *Wnt5a* and its transcription factor Twist are markedly over-expressed in TNBC but not luminal breast cancer cells. We also discovered that constitutively activated NF-kB in TNBC sustains prolonged activation of pro-inflammatory cytokines, enabling rapid spread (metastasis) of TNBC tumors. *Notably*, the functions of both transcription factors Twist and NF-kB in gene activation require lysine acetylation, which signs to activate the transcriptional machinery in chromatin. This chemical modification enables them to recruit the major transcriptional regulatory co-activator proteins to coordinate target gene activation in the human genome. In this study, we will investigate the underlying mechanism of gene activation in TNBC. We are developing novel small molecule compounds to render the transcription factor/co-activator activity in gene activation, a key function required for the prolonged expression of inflammatory cytokines that fuel TNBC cells proliferation and spreading. Our study should have a major impact on new targeted therapy development to fight against the aggressive TNBC.

15. SUBJECT TERMS

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1. Introduction

Breast cancer is the most commonly occupying cancer among women. While great stride is made in the recent years in disease diagnosis and treatment, we still don't have effective means to treat a major sub-population of metastatic breast cancer patients, particularly those who suffer from *triple-negative breast cancer* (TNBC). The average time to live after documentation of metastasis is only about two years. Unlike other subtypes, TNBC lacks the expression of three receptors: estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (Her2/neu), hence the name. The available treatments targeting these receptors do not work for TNBC patients. Studies show that inflammatory genes drive rapid progression of TNBC, and epithelial-mesenchymal transition (EMT), a process of massive cell movement required for morphogenesis in embryonic development, is responsible for cancer cell invasion and metastasis. The major challenge in TNBC research is to identify the factors within the cell that initiate and promote tumor metastasis. Our goal is to determine the role of gene transcriptional regulation in the development of metastatic TNBC. We focus on the function of lysine acetylation in gene activation to attain both mechanistic insights and rational design of small molecules that modulate the acetyl-lysine binding activity of the bromodomain (BrD), which function was first discovered by the M.-M. Zhou lab (*Nature,* 1999) (PI of this project). BrDs are embedded in many transcription-associated proteins such as the BET (bromo and extra-terminal domain) proteins important for transcriptional activation of pro-inflammatory and oncogenesis genes in TNBC. Our recent study between the labs of Drs. M.-M. Zhou and B.P. Zhou (an expert investigator on TNBC; also Partnering PI of this project) has attributed rapid tumor growth and metastasis of TNBC cells to tumor initiating, undifferentiated stem cell properties, and to over-activation of oncogenes (*Cancer Cell,* 2014). We show that a new class of BrD inhibitors (BrDis), we developed, effectively down-regulate expression of cancer stem cell (CSC) factors, inhibit oncogenic gene expression, and suppress rapid growth and invasion properties of TNBC cells.

We hypothesize that the inflammatory, EMT and CSC properties of TNBC tumor are caused and fueled by transcriptional over-activation of inflammatory and oncogenic genes; chemical inhibition of such aberrant transcriptional activities can circumvent the aggression of metastatic TNBC tumor. To reach the goal of our proposed study, we will achieve the three Specific Aims: (1) Determine the role of BET proteins in gene transcriptional activation in TNBC; (2) Develop selective BrD inhibitors targeting oncogene-activation; and (3) Characterize the mechanism of the transcriptional program in TNBC cells.

EMT and CSC properties play a critical role in invasion, drug resistance, and tumor recurrence and are often associated with poor prognosis in TNBC patients. Our findings will contribute greatly toward the understanding of induction of EMT at metastasis. Our study also explores the therapeutic potential of targeting this initiating event for the treatment of metastatic breast cancer.

2. Keywords

BET – bromodomain and extra-terminal domain

BLBC – basal-like breast cancer

BrD – Bromodomain

ChIP – chromatin immunoprecipitation

ChIP-seq – chromatin immunoprecipitation sequencing

CSC – cancer stem cell

EMT – epithelial-mesenchymal transition

ER – estrogen receptor

FA – fluorescence anisotropy

FACS – flow cytometry analysis

Her2/neu – human epidermal growth factor receptor 2

ITC – isothermal titration calorimetry

NMR – nuclear magnetic resonance

PR – progesterone receptor

RNA-seq – RNA sequencing

TAMs – tumor-associated macrophages

TMA – tissue microarray

TNBC – triple-negative breast cancer

3. Accomplishments

3.1. What were the major goals of the project?

In this past 3 years of this project, we have focused our efforts in this study as outlined in the major Tasks 1-3 of our research proposal.

Task 1:

a. Determine binding specificity of the BrDs of the BET proteins to lysine-acetylated peptides derived from histones and major transcription proteins including Twist, NF-kB and STAT3.

b. Define the molecular basis of the BET BrDs' selective interactions with effector proteins through structure-guided analysis, and determining the key residues using site-directed mutagenesis.

c. Validate the selective molecular interactions of the BET BrDs with transcription proteins in luminal and basal-like breast cancer cell lines, with and without treatment of new BET BrD inhibitors.

Task 2:

a. Design and synthesize new diazobenzene analogs to optimize lead compounds with high affinity (K_d <100 nM) and selectivity (>100:1 for a target over closely related proteins). This is an iterative process, and is coupled to **task 2.2b-c** and **task 3.1a-c**.

b. Determine the detailed molecular basis of ligand recognition by the BET BrDs by obtaining SAR data of lead series, and by solving new crystal structures of new ligands bound to BET BrDs.

c. Validate the cellular efficacy (EC_{50} <1 μM) of new BrD inhibitors in multiple TBNC cell lines.

Task 3:

a. Elucidate BRD4 functions in EMT and CSC properties as well as tumorigenicty of TNBC cells *in vitro* and *in vivo* using the newly developed selective BrD inhibitors.

b. Identify direct target genes of BRD4 in TNBC cell lines through ChIP-seq and RNA-seq analysis.

c. Determine the transcriptional expression levels of target genes of BRD4 in human TNBC samples.

3.2. What was accomplished under these goals?

We have accomplished the overall goals of this collaborative study between Dr. M.-M. Zhou's Lab at Icahn School of Medicine at Mount Sinai and Dr. B.H. Zhou's Lab at Kentucky University College of Medicine in the past three years, as described by three main Tasks listed above. In particular, Dr. M.-M. Zhou's lab has performed comprehensive structural and biochemical analyses of interactions of the bromodomains of BET proteins, particularly BRD4 with lysine-acetylated histone H4 and transcription factors Twist, NF-kB, STAT3, and FOXO3a, as well as BRD2 with STAT3, and validated these molecular interactions of BET proteins in gene transcription in cells. The new structure-function knowledge of the BET bromodomains has aided Dr. M.-M. Zhou lab in structure-based rational design and synthesis of new chemical inhibitors for BET BrDs. At the same time, Dr. B.H. Zhou's lab has performed detailed functional characterization of BET proteins interactions with histones and key transcriptional factors using the new structural insights generated in Dr. M.-M. Zhou lab's study. Additionally, Dr. B.H. Zhou's lab has investigated the mechanistic linkage between TNBC, obesity and inflammation, which is helpful to our ongoing efforts in the development of novel targeted therapy for TNBC. Below, we highlight the key findings of our studies.

(A) Structural Mechanism of BET Bromodomain Recognition of Histones and Transcription Factors

Studies from us and other show that despite their high sequence similarity, the tandem bromodomains of BET proteins (**Figure 1A**) have distinct functions in directing lysine-acetylation-mediated protein-protein interactions in regulation of gene transcription in chromatin. Specifically, the first bromodomain (BD1) of BRD4 tends to be dedicated to binding to lysine-acetylated histone H4 at K5/K8 (H4K5ac/K8ac), a transcriptional activation mark, whereas the second bromodomain (BD2) is functionally versatile and engaged in recruitments of transcription factors for *cis*-regulatory enhancer assembly and cyclin T1 of pTEFb for phosphorylation of RNA PolII and activation of transcription elongation (**Figure 1B**). To understand the molecular basis of BET BrDs' interactions with histones and transcription factors/co-factors, we have solved several 3D structures of the bromodomains of BRD4 and BRD2 in complex with lysine-acetylated peptides derived from histone H4, NF-kB, Twist, FOXO3, and STAT3 using heteronuclear multidimensional NMR spectroscopy methods (**Figure 1C-E**). We have further

performed comprehensive *in vitro* biochemical and cell-based characterization of binding specificity of the BET bromodomains in the context of gene transcription in chromatin. The salient features about the bromodomains of the BET proteins emerging from our studies are following: **(1)** the BET bromodomains are distinct from other members of the bromodomain family in that they prefer to bind di-lysine acetylation sites that are close to each other in target protein sequence. One acetylated-lysine acts to anchor BrD binding through hydrogen-bonding interaction with highly conserved Asn140 in BRD4-BD1 or Asn433 in BD2, whereas the other acetylated-lysine reinforces BrD binding through mostly hydrophobic and aromatic interactions with residues in the target protein (**Figure 1C,D**). The latter is manifested by BRD2-BD2 recognition of Phe89 in STAT3-K87ac site (**Figure 1E**); **(2)** BD1 prefers binding to H4K5ac/K8ac; **(3)** His437 in BRD4-BD2, one of few residues in the Kac binding site different between BD1 and BD2 (corresponding Asp144 in BRD4-BD1), is a determinant residue for target transcription factor binding specificity for BD2 over BD1. *Additionally*, our structural studies have aided our recent discovery of the distinct functions of BRD4 and BRD2 in gene transcription in Th17 cells in that BRD2 acts as a chromatin regulator with CTCF/cohesin complex for enhancer assembly, whereas BRD4 functions as a transcription co-activator by recruiting p-TEFb to phosphorylate RNA PolII and activate transcription elongation (Cheung*, Mol. Cell,* 2017). Collectively, the new structural mechanism of the BET bromodomains in recognition of histones and transcription factors emerging from our studies is guiding us to develop selective bromodomain inhibitors in blocking transcriptional expression of oncogenes in the progression of metastatic breast cancer, particularly TNBC.

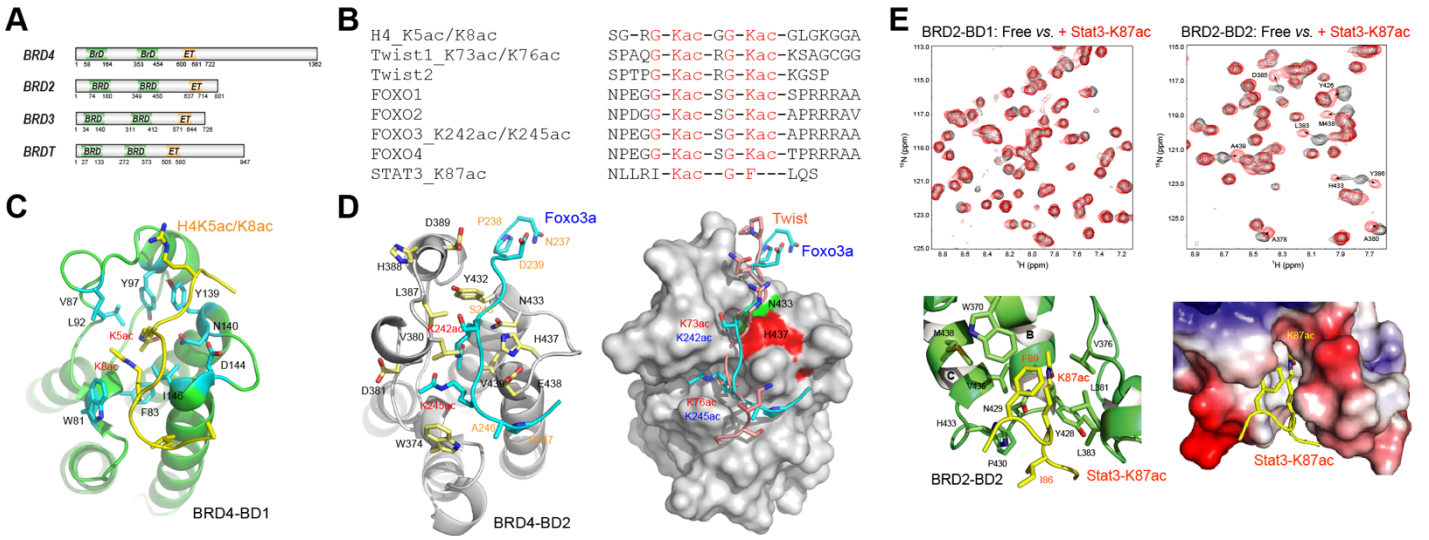

Figure 1. Structural mechanism of BET BrDs' recognition of lysine-acetylated histone H4 and transcription factors. (A) Domain organization of BET proteins. **(B)** Sequence alignment of di-acetylation sites in histone H4, Twist, FOXO family transcription factors and STAT3. **(C)** 3D structure of BRD4-BD1 in complex with H4K5ac/K8ac peptide. **(D)** NMR 3D structure of BRD4-BD2 bound to FOXO3-K242ac/K245ac peptide (cyan). *Right*, superimposition of FOXO3a and Twist1 peptides when bound to BRD4-BD2. **(E)** Upper panel, 2D ^{15}N-HSQC spectra of Brd2-BD1 or BD2 illustrating changes of the protein backbone amide resonances in the free form (black), and in the presence of Stat3-K87ac peptide (red). Lower panel, 3D NMR structure of BRD2-BD2 bound to Stat3-K87ac peptide (yellow), illustrating Stat3-K87ac recognition by the key residues at the Kac binding site. *Right*, electrostatic potential representation of BRD2-BD2 depicts Stat3-K87ac recognition in the Kac binding pocket.

(B) Structure-Guided Design of New BrD Inhibitors for the BET Proteins

Guided with our structural insights of BRD4 BrD/ligand recognition, we conducted several rounds of design, synthesis and structure-activity relationship (SAR) characterization of diazobenzene-based BrD inhibitors in an effort to develop potent and selective BrD inhibitors for the BrDs of BRD4. Specifically, our lead compound MS436, through a set of water-mediated interactions, exhibits low nanomolar affinity (K_i of ~50 nM) with clear preference for BD1 over BD2 of BRD4 (**Figure 1A,B**). We showed that MS436 effectively inhibits BRD4 activity in NF-κB-directed production of nitric oxide (**Figure 1C**) and pro-inflammatory cytokine interleukin-6 (IL-6) in murine macrophages known to promote pro-inflammatory activity in TNBC tumor development. We further designed new compounds by replacing the diazo-bridge with stable linking moieties such as a carbon-carbon double or triple bond. A double carbon-carbon bond linked lead MS255 retains affinity to BRD4 BrDs and inhibits NF-kB transcriptional activity as measured in nitric oxide release in mouse macrophage RAW264.7 cells (**Figure 1B,C**). Our *in vivo* mouse PK study showed that MS255 has improved bioavailability and higher circulating plasma level of 5 μM with subcutaneous dosing up to 8 hours as compared to 1.5 hours for MS436. Of our new-generation diazobenzene compounds, MS611 exhibits highly promising 100-fold selectivity for BD1

over BD2 of BRD4 (**Figure 2B**). Our new crystal structure of MS611/BRD4-BD1 complex reveals that cyano-pyridine group, *para*- to sulfonamide, interacts directly with unique residues Lys91 and Asp145 in BD1, explaining MS611 superior selectivity (**Figure 2D**). We further observed that MS611 has much better beneficial effects than pan-BET BrD inhibitors such as JQ1 in modulating gene transcription in biological processes. Currently, we are evaluating and optimizing *in vivo* PK properties of our new leads with different administration routes of intravenous, subcutaneous injection, and oral gavage. These studies are important and relevant for our efforts of developing these compounds into new therapeutic agents for disease treatment.

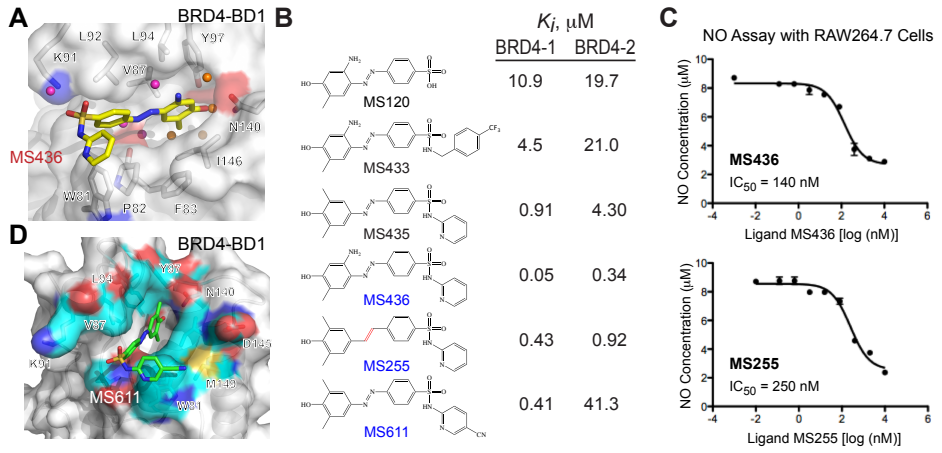

Figure 2. Structure-based development of selective BRD4 BrD inhibitors. (**A**) Crystal structures of BRD4-BrD1 bound to a lead BrDi MS436 (yellow); **(B)** Binding affinity of diazo-benzene analogs against two BRD4 BrDs measured using a fluorescence anisotropy assay with a FITC-labeled MS417 as an assay probe; (**C**) Inhibition of NF-kB-directed NO activation and release in mouse macrophage RAW264.7 cells upon the treatment of MS436 and MS255 in a concentration-dependent manner. **(D)** Crystal structures of BRD4-BrD1 bound to a lead BrDi MS611 (green).

(C) BRD4 Inhibition is Synthetic Lethal with Dox Treatment by Blocking Immune Survival Response

In our recent study, we discovered that doxorubicin, a commonly used chemotherapeutic agent for TNBC treatment, activates immune response and DNA damage repair genes that collectively act as a major barrier preventing TNBC tumor cells from apoptosis. *Notably*, we observed that BRD4 inhibition produces synthetic lethality with Dox treatment in inducing HCC1806 TNBC cell apoptosis much more so than Dox combination treatment with PARP1/2 inhibitor Olaparib (**Figure 3A**). Given that PARP inhibition works best in BRCA1 deficient cells, we tested and found that Dox+MS417 is just as effective as, if not more than Dox+Olaparib combination in inducing cell apoptosis with BRCA1-defective SUM149PT tumor cells in a dose-dependent manner (**Figure 3A**). In a mouse xenograft study using nude mice injected with HCC1806 cells, we found that the Dox+MS417 combination treatment exerted much more profound tumor growth inhibition than single agent or placebo treatment, as assessed by tumor volume and weight (**Figure 3B**). Finally, the mice of the combination treatment group showed markedly reduced protein level of RAD51 (**Figure 3C**), as well as other genes important for immune survival response including chemokines in tumor tissues as compared to the single agent treated mice, or the control group mice. Collectively, these results suggest Dox and BRD4 inhibition combination as a new epigenetic chemotherapy that overcomes drug resistance for TNBC treatment.

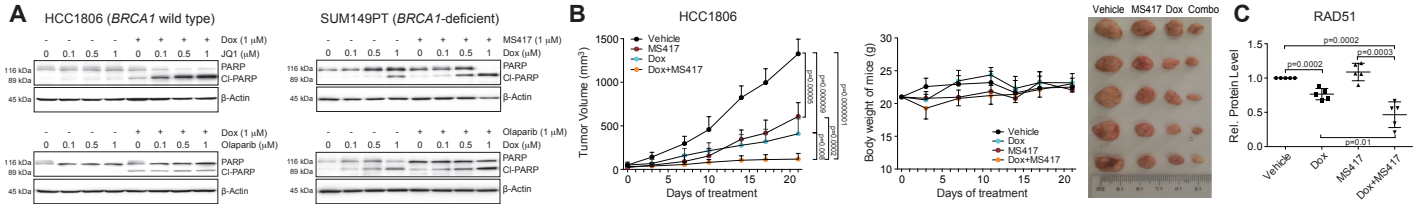

Figure 3. Doxorubicin and MS417 combination treatment synergizes *in vivo* inhibition of TNBC tumorigenesis in mice. (A) Western blot showing PARP and cl-PARP levels in HCC1806 cells (BRCA1 wild type) or SUM149PT cells (BRCA1 deficient) treated with Dox or in combination with BRD4 inhibitor (JQ1/MS417) or PAPP1/2 inhibitor (Olaparib). **(B)** HCC1806 cells ($4x10^6$) were injected into the mammary fat pad of nude mice. When tumors in the mice reached 50 mm^3, the mice were divided into four groups and treated with vehicle (PEG400, i.p.), Dox (0.8 mg/kg, intratumor injection), MS417 (40 mg/kg, i.p.), or the drug combination, respectively. The error bars indicate SD from five mice in each group. The p value was calculated using Sidak's multiple comparisons test. **(C)** Effects of Dox, MS417 or combination treatment on RAD51 protein level in tumor tissues from mice in the xenograft study as indicated.

(D) Twist Regulates ATX and LPAR1 Expression

The rapid tumor growth as well as aggressive metastasis of TNBC heavily relies on aberrant up-regulation of pro-oncogenic inflammatory pathways. Elucidation of the transcriptional program regulated by Twist helps us better understand the mechanistic linkage between TNBC and obesity. From numerous studies, we learn that ATX a secreted enzyme (encoded by *ENPP2*) produces most of the extracellular lysophosphatidic acid (LPA),

which signals through its receptors (LPAR1-6) to mediate a wide range of inflammatory processes including wound healing, fibrosis and metastasis. Aberrant expression of ATX and LPARs has been linked to invasion, migration and metastasis of many types of cancers, including TNBC. Our current study indicates that Twist activation intensifies the inflammatory ATX-LPAR1 signaling to promote the development and progression of obesity-associated TNBC (see **Figure 4a**). We generated stable human normal breast epithelial cell lines MCF10A and HMLE, as well as luminal breast cancer cell line T47D with ectopic overexpression of Twist, and performed cDNA microarray screen to identify potential Twist target genes. It was revealed that the mRNA levels of Autotaxin (ATX) and LPAR1 were dramatically increased upon Twist over-expression (**Figure 4b**). Consistently, we found that ATX and LPAR1 are highly expressed in TNBC cells, and their expression correlates with that of Twist (**Figure 4c**). Notably, aberrant expression of ATX and LPARs has been linked to invasion, migration and metastasis of many types of cancers, including TNBC. Importantly, AT is a major source for the synthesis and secretion of ATX; dysregulation of ATX level/activity is involved in diet-induced obesity, with the underlying mechanism remaining contentious. Currently, we focus our efforts to elucide in details the mechanistic features underlying Twist-ATX-LPAR1 signaling axis in the development of TNBC. We are addressing questions including the function of Twist in regulation of ATX and LPAR1 expression, the role of Twist-ATX-LPAR1 axis during TNBC cell-adipocyte crosstalk in cells and in TNBC tumor growth *in vivo*.

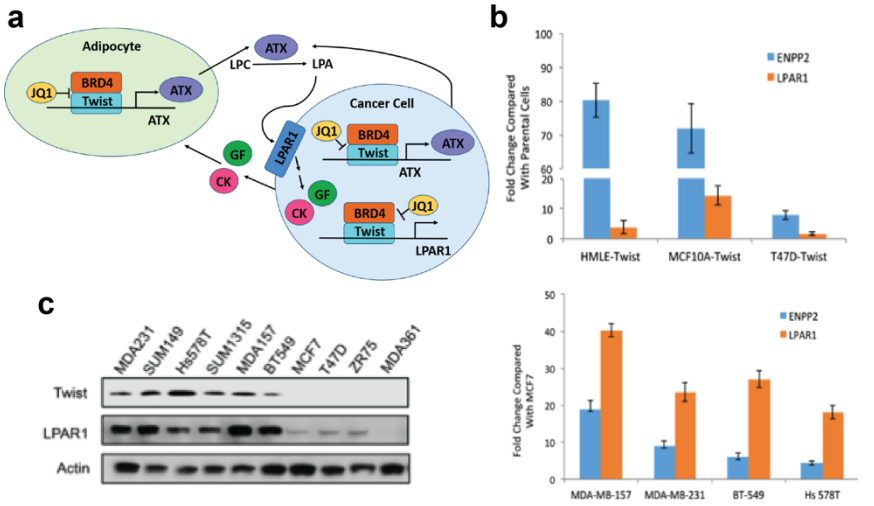

Figure 4. The role of Twist-ATX-LPAR1 signaling axis during TNBC cell-adipocyte crosstalk. (a) Scheme depiction of the Twist-ATX-LPAR1 signaling axis in TNBC. **(b)** ENPP2 and LPAR1 are potential target genes of Twist. Upper panel, Twist overexpression increased mRNA levels of ENPP2 and LPAR1. Lower panel, fold change of ENPP2 and LPAR1 mRNA levels in TNBC cells compared to MCF7 cells. **(c)** Western blotting showing expression of Twist and LPAR1 in TNBC cells and luminal subtype cells.

3.3. What opportunities for training and professional development has the project provided?

In the 3-year funding period, the professional development of both Drs. Ming-Ming Zhou and Binhua P. Zhou was further strengthened and broaden as indicated in their active participation of breast cancer-related grant review at the NCI, DoD and Komen Cancer Foundation, as well as professional activities in reviewing scientific journals, and the presentation at several meetings and institutes, as shown below:

A. Grant Review:

Ming-Ming Zhou

2014 - 19 Regular Member, NIH - "Macromolecular Structure and Function B" (MSFB)
2015 Worldwide Cancer Research
2016 National Science Foundation
2016 NIH - New Innovator Award
2017 NIH - *"Cancer Drug Development & Therapeutics"* (CDDT)
2017 NIH/NIAID - Special Emphasis Panel, ZAI1 CB-A(M1) 1
2017 Wellcome Trust, UK
2017 Breast Cancer Alliance
2017 NIH - Chair, Member-Conflict /Special Emphasis Panel (ZRG1 BCMB-X(02)

Binhua P. Zhou

2015 - 19 Regular member, TPM study section, National Cancer Institute (NCI)
2015 Reviewer, DOD BRCP Breakthrough Award Panel (PBY3)
2015 Reviewer, Mary Kay Ash Foundation for Cancer Research Grants, Dallas, TX
2016 Reviewer, Susan G. Komen Foundation

2016 Reviewer (ad hoc), Susan G. Komen Foundation
2017 Reviewer, DOD BRCP PYB-4 study section
2018 Reviewer, DOD BRCP PYB-2 study section
2015-19 Standing member, TPM study section, National Cancer Institute (NCI)

B. Editor/Service on Editorial Boards:

Ming-Ming Zhou

2009 - Editorial Board, *Journal of Molecular Cell Biology*
2010 - Editorial Board, *ACS Medicinal Chemistry Letters*
2010 - *Faculty of 1000* on *"Structure, and Transcription and Translation"*
2012 - Editorial Board, *Journal of Cancer Immunology*
2015 Editor, *"Histone Recognition", Springer*
2015 Co-Organizer, 2015 FASEB Research Conference on *"HDACs, Sirtuins and Reversible Acetylation in Signaling and Disease",* (Co-Organizer, David Sinclair)
2016 Guest Editor (with Steven Smith), *"Drug Discovery Today: Technologies", Elsevier*
2017 Guest Editor (with Evripidis Gavathiotis), *"Current Opinion in Chemical Biology", Elsevier*

Binhua P. Zhou

2010 - Editorial board member: *Scientific Reports*, *Cancer Hallmarks, Journal of Cancer Science & Therapy, American Journal of Cancer Biology, International Journal of Biological Chemistry*
2011 - Editor, *Cancer Reports, Pancreatic Disorders & Therapy*
2015 - Associate Editor, *Molecular and Cellular Oncology* (sections of Frontiers in Cell and Developmental Biology and Oncology)
2015 - Consulting Editors, *JCI Insight*
2015 - Editorial board member: *Scientific Reports*,
2015 Guest Editor, Special issue of "Epithelial-mesenchymal Transition in Cancer Progression and Metastasis", *Cancer and Metastasis Reviews*

3.4. How were the results disseminated to communities of interest?

We have been disseminating the results of our study to the research community through invited talks at the universities and scientific conferences in the past 3 years, as well as publications:

A. Presentations

Ming-Ming Zhou

03/05/2015 University of Iowa, Department of Biochemistry, Iowa City, Iowa*, "Epigenetic Mechanism of Gene Transcription in Biology and Disease"*
03/26/2015 Vanderbilt-Ingram Cancer Center, Vanderbilt University School of Medicine, Nashville, TN, *"From Epigenetic Mechanism to Targeted Therapy"*
04/01/2015 The Children's Hospital of Philadelphia, The University of Pennsylvania School of Medicine, Philadelphia, PA, *"From Epigenetic Mechanism to Targeted Therapy"*
05/19/2015 University of California San Francisco, Pharmacology and Pharmacogenomics Program, San Francisco, CA, *"From Epigenetic Mechanism to Targeted Therapy"*
08/19/2015 2015 FASEB Research Conference on *"HDACs, Sirtuins and Reversible Acetylation in Signaling and Disease",* Co-Organizer, Germany (with D. Sinclair), *"Distinct Roles of BET Proteins in Gene Transcription"*
12/16/2015 PacifiChem 2015, Symposium on *"Frontiers in Chromatin Biology and Chemical Epigenetics/ Epigenomics,"* Honolulu, Hawaii, *"From Epigenetic Mechanism to Targeted Therapy"*
03/01/2016 New York Genome Center, NY Cancer Genomics Research Network, NY, *"From Epigenetic Structural Mechanism to New Therapy"*
03/13/2016 2016 ACS National Meeting, Symposium on *"Bromodomain Inhibition: BETs and Beyond",* San Diego, CA, *"From Epigenetic Mechanism to Targeted Therapy"*
04/01/2016 University of Florida College of Medicine, Center for Epigenetics, Gainesville, FL, *"From Epigenetic Mechanism to Targeted Therapy"*
05/03/2016 Mayo Clinic, Department of Biochemistry & Molecular Biology, Rochester, MN, *"From*

Epigenetic Mechanism to New Targeted Therapy"

06/11/2016 Chemical Biology Session at the ACS 44th Middle Atlantic Regional Meeting (MARM 2016), The College of Mount Saint Vincent in Riverdale, NY, *"From Epigenetic Structural Mechanism to Targeted Therapy"*

11/09/2016 University of Wisconsin-Madison, Cancer Biology Seminar Series, Madison, WI, *"From Epigenetic Mechanism to Targeted Therapy"*

12/22/2016 The First Bethune Hospital, Jilin University School of Medicine, Changchun, China, *"New Structural Mechanisms of Epigenetic Control of Gene Transcription"*

02/16/2017 Purdue University Cancer Center, West Lafayette, IN, *"From Epigenetic Structural Mechanism to Targeted Therapy"*

04/24/2017 Hong Kong University, School of Biomedical Sciences, Hong Kong, China, *"From Epigenetic Mechanism to Targeted Therapy"*

04/19/2017 2017 Health & Bio Technology Summit, New York, NY, *"New Epigenetic Therapy for Cancer and Inflammatory Disorders"*

05/01/2017 The Samuel Waxman Cancer Research Foundation, New York, NY, *"Modulating Transcription Repression for Targeted Epigenetic Cancer Therapy"*

05/10/2017 NewYorkBio 2017 Annual Conference, New York, NY, *"Developing New Epigenetic Cancer Therapy"*

08/08/2017 2017 FASEB Research Conference on *"Reversible Acetylation in Health and Disease",* Big Sky, Montana, *"New Kid on the Block: a Role of New Histone Modifications in Gene Transcription"*

10/06/2017 Plenary Lecture, the ICB&DD 11th Annual Symposium on "Frontiers in Chemical Biology and Drug Discovery", Institute of Chemical Biology & Drug Discovery, Stony Brook University, Stony Brook, NY, *"From Epigenetic Mechanism to Targeted Therapy"*

10/30/2017 Sinai Innovations, Icahn School of Medicine at Mount Sinai, New York, NY, *"Synergizing Scientific Innovation and Discovery of New Medicines"*

01/18/2018 NY Academy of Sciences, *"New York Structural Biology Discussion Group"*, NYC, *"From Epigenetic Mechanism to Targeted Therapy"*

04/30/2018 The Samuel Waxman Cancer Research Foundation, New York, NY, *"Relieving Transcription Repression as Targeted Epigenetic Cancer Therapy"*

Binhua P. Zhou

03/14/2015 Department of Biochemistry, University of Florida, Gainesville, FL

03/05/2015 Elkin lecture, Winship Cancer Center, Emory University School of Medicine, Atlanta, GA

04/272015 Department of System Biology, University of Pittsburgh, Pittsburgh, PA

05/212015 Department of Cancer Biology, Wake Forest University School of Medicine, Winston-Salem, NC

05/10/2015 Karmanos Cancer Institute, Detroit, Michigan

08/12/2015 Cancer Biology Program, City of Hope, Los Angeles, CA

08/28/2015 Houston Methodist Research Institute/Weill Medical College at Cornell University, Houston, TX

11/12/2015 Stephenson Cancer Center, University of Oklahoma Health Science Center, Oklahoma City, OK

12/02/2015 Breast Cancer Research Program, University of California, Los Angeles, CA

12/18/2015 Department of Medicine & Biochemistry and Molecular Biology, Mayo Clinic, Rochester, MN

04/13/2016 Department of Experimental Therapeutics, MD Anderson Cancer Center, Houston; *"Epithelial-mesenchymal transition in Breast Cancer Progression and Metastasis"*

06/12/2016 The Wistar Cancer Institute, Philadelphia, PA, *"Role and Regulation of Epithelial-mesenchymal transition in Breast Cancer"*

11/12/2016 Stanley S. Scott Cancer Center, Louisiana State University, New Orleans, LA, *"Distinct Roles of Snail and Twist in Epithelial-mesenchymal transition"*

02/23/2017 Department of Pharmacology, University of California at San Diego, CA, *"Epithelial-mesenchymal transition in Breast Cancer Progression and Metastasis"*

03/14/2017 Departments of Pathology, UT Southwestern Medical Center, Dallas, TX, *"Distinct Roles of Snail and Twist in Breast Cancer Progression and Metastasis"*

04/20/2017 Distinguished Scientist Speaker, University of Southern Alabama Mitchell Cancer Institute, Mobile, AL, *"Role and Regulation of Epithelial-mesenchymal transition in Breast Cancer"*

10/06/2017 Indiana University Melvin and Bren Simon Cancer Center, Indianapolis, IN, *"Epithelial-mesenchymal transition in Breast Cancer Progression and Metastasis"*

12/04/2017 Department of Cancer Biology, University of Cincinnati College of Medicine, Cincinnati, OH, *"Distinct Roles of Snail and Twist in Breast Cancer Progression and Metastasis"*
03/01/2018 Department of Cancer Genetics and Genomics, Roswell Park Cancer Institute, Buffalo, NY, *"Epithelial-mesenchymal transition in Breast Cancer Progression and Metastasis"*
05/31/2018 UM Comprehensive Cancer Center, University of Michigan, Ann Arbor, MI, *"Epithelial-mesenchymal transition in Breast Cancer Progression and Metastasis"*
12/07/2018 University of Alabama at Birmingham Comprehensive Cancer Center, Birmingham, AL

B. Publications relevant to this project

Hu, Y., Zhou, J., Ye, F., Xiong, H., Peng, L., Zheng, Z., Xu, F., Cui, M., Wei, C., Wang, X., Wang, Z., Zhu, H., Lee, P., **Zhou, M.-M.,** Jiang, B., & Zhang, D.Y. (2015) BRD4 Inhibition Inhibits Tumor Growth and Metastasis in Colorectal Cancer. ***International Journal of Molecular Sciences,*** 16(1): 1928-48.

Smith, S.G., & **Zhou, M.-M.** (2015) The Bromodomain as the Acetyl-Lysine Binding Domain in Gene Transcription in *"Histone Recognition"* (M.-M. Zhou, Ed.) Springer (DE), Heidelberg, Germany, pages 1-26.

Stratikopoulos, E.E., Dendy, M., Szabolcs, M., Khaykin, A.J., Lefebvre, C., **Zhou, M.-M.,** & Parsons, R. (2015) Kinase and BET Inhibitors Together Clamp Inhibition of PI3K Signaling and Overcome Resistance to Therapy. ***Cancer Cell,*** 27, 837-851.

Sharma, R., & **Zhou, M.-M.** (2015) Partners in Crime: The Effects of Tandem Modules in Gene Transcription. ***Protein Sciences,*** 24(9):1347-1359.

Zhang, G., Smith, S.G., & **Zhou, M.-M.** (2015) Discovery of Chemical Inhibitors of Human Bromodomains. ***Chemical Reviews***. 115(21):11625-68.

Smith, S.G., & **Zhou, M.-M.** (2015) The Bromodomain: A New Target in Emerging Epigenetic Medicine. ***ACS Chem. Biol.*** Epub 2015 Nov 23. PMID: 26596782.

Cheung, K.L., Zhang, F., Jaganathan, A., Sharma, R., Zhang, Q., Konuma, T., Shen, T., Lee, J.-Y., Ren, C.Y., Chen, C.-H., Lu, G., Olson, M.R., Zhang, W., Kaplan, M.H., Littman, D.R., Walsh, M.J., Xiong, H., Zeng, L., & **Zhou, M.-M.** (2017) Distinct Roles of Brd2 and Brd4 in Potentiating the Transcriptional Program for Th17 Cell Differentiation. ***Molecular Cell,*** 65(6): 1068-1080.

Cheung, K.L., Lu, G.M., Sharma, R., Vincek, A.S., Zhang, R.H., Plotnikov, A.N., Zhang, F., Zhang, Q., Ju, Y., Hu, Y., Zhao, L., Han, X., Meslamani, J., Xu, F., Jaganathan, A., Shen, T., Zhu, H., Rusinova, E., Zeng, L., Zhou, J.C., Yang, J.C., Peng, L., Ohlmeyer, M., Walsh, M.J., Zhang, D.Y., Xiong, H.B., & **Zhou, M.-M.** (2017) Selective BET Bromodomain Inhibition Blocks Th17 Cell Differentiation and Ameliorates Colitis in Mice. ***PNAS.*** 114(11): 2952-2957.

Gavathiotis, E., & Zhou, M.-M. (2017) Editorial Overview: Chemical Genetics and Epigenetics. ***Curr. Opin. Chem. Biol.,*** vol. 39, pages vi-vii. PMID: 28801102.

Zaware, N., & Zhou, M.-M. (2017) Chemical Modulators for Epigenome Reader Domains as Emerging Epigenetic Therapies for Cancer and Inflammation. ***Curr. Opin. Chem. Biol.,*** 39:116-125. PMID: 28689146.

Conrad, R.J., Fozouni, P., Thomas, S., Sy, H., Zhang, Q., Zhou, M.-M., Ott, M. (2017) The Short Isoform of BRD4 Promotes HIV-1 Latency by Engaging Repressive SWI/SNF Chromatin-Remodeling Complexes. ***Molecular Cell,*** 67(6): 1001-1012. PMID: 28844864.

Wang Y, Liu J, Ying X, Lin PC, and **Zhou BP***, (2016) Twist-mediated Epithelial-mesenchymal Transition Promotes Breast Tumor Cell Invasion via Inhibition of Hippo Pathway. ***Scientific Reports*** 6:24606.

Wu Y, Wang Y, Lin Y, Liu Y, Wang Y, Jia J, Singh P, Chi Y-I, Wang C, Dong C, Li W, Tao M, Napier D, Shi Q, Deng J, Evers BM, and **Zhou BP***, (2017) Dub3 Inhibition Suppresses Breast Cancer Invasion and Metastasis by Promoting Snail1 Degradation. ***Nature Communications*** 8:14228.

Lin Y, Wang Y, Shi Q, Deng J, Evers BM, and **Zhou BP*** Wu Y*, (2017) Stabilization of the transcription factors slug and twist by the deubiquitinase dub3 is a key requirement for tumor metastasis, ***Oncotarget*** 8:75127-75140

Wang J, Ye Q, Cao Y, Guo Y, Huang X, Mi W, Liu S, Wang C, Yang H.-S., **Zhou BP**, Evers BM, She Q.-B, (2017) Snail determines the therapeutic response to mTOR kinase inhibitors by transcriptional repression of 4E-BP1, ***Nature Communications*** 8(1):2207

Liu J, Wu Y, Deng J, Lin Y, Wang C, Liu J, Lin PC, Evers BM, Zhou MM*, Shi J*, **Zhou BP***, (2018) Targeting the BRD4/FOXO3a/CDK6 axis Sensitizes AKT Inhibition in Luminal Breast Cancer, Nature Communications, *minor revision.*

3.5. What do you plan to do during the next reporting period to accomplish the goals?
We are continuing our efforts in dissecting the molecular mechanism of gene transcriptional regulation or mis-regulation underlying metastatic breast cancer beyond this funding period. Because the major incompletely answered questions still remain on how lysine acetylation-mediated protein-protein interactions work in favor of transcriptional expression of oncogenes that promote and sustain rapid progression and spreading of advanced breast cancer, our current focus is centered on exploring new therapeutic approaches to block disease advance, which are based on our new deep functional and mechanistic understanding of disease-associating transcription factors and chromatin regulator proteins in regulation or mis-regulation of gene transcription in the disease state. *In summary,* we have accomplished the overall goals in the past 3-year funding period of this project by addressing nearly all the proposed studies as stated in the major tasks in the Statement of Work. We will continue our efforts to achieve the ultimate goal of developing new targeted epigenetic therapy for safe and more effective treatment for metastatic breast cancer including the devastating triple-negative breast cancer that currently still lacks targeted therapy.

4. Impact
Triple-negative breast cancer (TNBC) is the most aggressive subtype of breast cancer that is associated with early metastasis to brain and lung, poor prognosis and short survival. About 240,000 women were diagnosed worldwide in 2012 with breast cancer, of which ~20-25% are of TNBC. TNBC disproportionally affects women of African and Hispanic descent, and occurs more often in younger women, affecting women as early as in their 20s. 80% of breast cancer in people with an inherited BRCA1 mutation is found to be TNBC. TNBC lacks expression of three receptors, i.e. estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (Her2/neu), hence its name. Most available breast cancer treatments target these receptors. Unfortunately, given their triple negative status, TNBC tumors generally do not respond to receptor-targeted treatments. Depending on the stage of its diagnosis, TNBC is very aggressive, highly metastatic, and much more likely to recur than other breast cancer subtypes. Currently, there is no targeted therapy for TNBC. The standard of care for TNBC is surgery with adjuvant chemotherapy and radiation therapy, which is not effective once the tumor is spread.

Recent studies suggest that TNBC is inflammation-associated cancer - its rapid tumor growth and metastasis is heavily dependent upon and fueled by markedly elevated transcriptional activation of pro-inflammatory cytokines and EMT program. As such, chemical inhibitors that target epigenetic proteins whose functions are required for over-expression of these oncogenes offer an exciting opportunity to develop a new targeted epigenetic therapy to fight triple-negative breast cancer. Therefore, the funding provided by the DoD Breast Cancer Breakthrough Award will greatly accelerate our ongoing efforts to test our hypothesis, and validate our novel lead chemical compounds as a potentially new targeted epigenetic therapy to fight against this aggressive and devastating disease.

5. Changes/Problems

Nothing to Report

6. Products

Nothing to Report

7. Participants & Other Collaborating Organizations

What individuals have worked on the project?
Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change."

Note: For the last 12 months: 03/15/2017 – 03/14/2018

Name:	Ming-Ming Zhou
Project role:	PI
Researcher identifier:	#1 at ISMMS
Nearest person months worked:	1
Contribution to project:	Directing the project
Name:	Chunyan Ren
Project role:	Biochemist /Postdoctoral Fellow
Researcher identifier:	#2 at ISMMS
Nearest person months worked:	10
Contribution to project:	Structural analysis of BET BrDs recognition of lys-acetylated histones and transcription factors, or small molecule inhibitors
Name:	Tsuyoshi Konuma
Project role:	Biochemist /Postdoctoral Fellow
Researcher identifier:	#3 at ISMMS
Nearest person months worked:	5
Contribution to project:	SAR study of BET BrD/ligand binding
Name:	Jamel Meslamani
Project role:	Structural Chemist /Postdoctoral Fellow
Researcher identifier:	#4 at ISMMS
Nearest person months worked:	1
Contribution to project:	Structure-based design and analysis of BRD4 BrDs
Name:	Binhua P. Zhou
Project role:	PI
Researcher identifier:	#1 UKSoM
Nearest person months worked:	1
Contribution to project:	Directing the project
Name:	Yuting Zhou
Project role:	Graduate Student
Researcher identifier:	#2 UKSoM
Nearest person months worked:	4
Contribution to project:	Conducted identification of the interaction of BDR4 with FOXO3a and characterization of the target gene CDK6 of the BRD4-FOXO3a complex.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

In the past 12 months, Drs. Ming-Ming Zhou and Binhua Zhou have some changes in their group's research grants, as listed below:

New Research Support

1R43DK113847-01A1 (mPIs: Q. Zhang, M.-M. Zhou) 09/01/2017 – 08/31/2018 0.6 cal. mon.
NIH/NIDDK $57,997/yr, d.c. (Zhou Lab)
"New Th17 Selective Immunomodulators for Inflammatory Disorders"
This project aims to develop new immunomodulators targeting Th17 cells in inflammatory disorders.

PI: M.-M. Zhou 07/01/2017 – 06/30/2018 0.3 cal. mon. (*no salary support)
The Samuel Waxman Cancer Research Foundation $15,000/yr, d.c.
"Modulating Transcriptional Repressor Sin3A for Targeted Epigenetic Cancer Therapy"
This project aims to develop small molecule modulators for Sin3A using structure-guided approaches.

P20 GM121327 (mPIs: St. Clair, D; Zhou, BP) 03/01/2017-12/31/2021 3.6 cal. mon.
NIH/NIGMS $2,220,230/yr, d.c
"University of Kentucky Center for Cancer and Metabolism"
Goals: To strengthen UK's cancer research enterprise by providing a thematically focused multidisciplinary infrastructure dedicated to defining the contribution of metabolism in the development and treatment of cancer and to use this novel multidisciplinary platform to develop promising early-stage investigators with enhanced skills in an exciting new area of cancer research.

8. Special Reporting Requirements

N/A

9. Appendices

3 papers as listed in 3.4B.