ZNF33A Promotes Tumor Progression and BET Inhibitor Resistance in Triple-Negative Breast Cancer

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In various malignant tumors, overexpression of ZNF33A (Krüppel-type zinc finger 33A) promotes carcinogenesis. Nevertheless, the biochemical role and clinical importance of ZNF33A in triple-negative breast cancer (TNBC) still need to be explored. In this study, ZNF33A expression was increased abnormally in TNBC patient tissues and cell lines, leading to a worse prognosis. Furthermore, ZNF33A promoted cell growth and facilitated the resistance of cancer cells to inhibitors of bromodomain and extraterminal domain (BET) in TNBC. Our findings indicated also that ZNF33A promotes the induction of c-Myc, the main reason for resistance to BET inhibitors in TNBC. In conclusion, ZNF33A may be a tumor growth—promoting factor associated with TNBC prognosis, and TNBC cells are sensitized by ZNF33A repression to BET inhibitors. (Am J Pathol 2022; 190: 1–12; https://doi.org/10.1016/j.ajpam.2022.06.010)
regulate the expression of oncogenes such as c-Myc.\textsuperscript{17} For instance, the most thoroughly characterized BET protein, BRD4, is crucial for the proliferation of colorectal cancer cells.\textsuperscript{18,19}

In the current study, the clinical significance and biological role of ZNF33A in TNBC cells were explored by assessing its expression in TNBC patient specimens. We also evaluated the role of ZNF33A in the sensitivity of small antitumor molecules and examined whether the resistance of cancer cells to BET inhibitors is mediated by ZNF33A via up-regulation of c-Myc expression.

Materials and Methods

Patient Samples

The TNBC and adjacent normal tissues (\(N = 62\)) used in this study were obtained from the First Affiliated Hospital of China Medical University along with complete clinicopathologic data (Table 1). All studies were approved by the Ethics Committee of China Medical University, and informed consent was obtained from all patients.

Cell Lines and Reagents

Human breast cancer cell lines (BT-594, MDA-MB-231, MDA-MB-468, BT-20, and MDA-MB-453) were procured from ATCC (Manassas, VA) and grown in Dulbecco’s modified Eagle’s medium (Invitrogen, Carslbad, CA) with 10\% defined fetal bovine serum (HyClone, Logan, UT), penicillin (100 U/mL), and streptomycin (100 \(\mu\)g/mL; Invitrogen). The cells were grown in a 5\% carbon dioxide atmosphere at 37\(^\circ\)C. Human healthy breast epithelial cell lines (MCF-10 A and HBL-100) were cultured in Dulbecco’s modified Eagle’s medium/F-12 media (Invitrogen) supplemented with 10\% defined fetal bovine serum, penicillin (100 U/mL), and streptomycin (100 \(\mu\)g/mL; Invitrogen), 20 ng/mL epidermal growth factor (Invitrogen), 100 ng/mL cholera toxin (Invitrogen), 0.5 mg/mL hydrocortisone (Invitrogen), and 10 \(\mu\)g/mL human insulin (MilliporeSigma, Burlington, MO). ZNF33A-KO MDA-MB-231 and BT-594 cells were generated as described previously with CRISPR/Cas9 using a single guide RNA sequence 5’-GTTGAGCAAGTCCGATTATTA-3’. In 2020, authentication of these cells was performed by genotyping and protein expression analysis by Western blot, and PCR was conducted to routinely check for contamination by Mycoplasma. Before drug treatment, cells were plated at a density of 20\% to 30\% in 12-well plates for 24 hours. Stocks of I-BET151, JQ1, OTX015, oxaliplatin, 5-fluorouracil, tametinib, palbociclib, olaparib, everolimus, azacitidine, and vorinostat were prepared in dimethyl sulfoxide (MilliporeSigma) and diluted in cell culture medium before their addition to cells.

| Table 1: Characteristics of Patients with TNBC |
|-----------------|-----------------|-----------------|
| Clinical parameter | Total patient no. (\(N = 62\)) | Percentage |
| Age at diagnosis (year) | Patient no. | Percentage |
| <45 | 25 | 40.3 |
| \(\geq 45\) | 37 | 59.6 |
| Lymph node metastasis | | |
| N0 | 21 | 33.9 |
| N1 | 41 | 66.2 |
| N2 | 0 | 0.0 |
| N3 | 0 | 0.0 |
| Stage | | |
| I | 24 | 38.7 |
| II | 36 | 58.1 |
| III | 2 | 3.2 |
| IV | 0 | 0.0 |

Transfection

Transfection of cell lines was performed with Lipofectamine 2000 (Invitrogen). Prior to this, V5-ZNF33A was cloned into the pCDNA3.1-V5 vector. Twenty-four hours before drug treatment, 200 pmol of ZNF33A-siRNA (sc-134182; Santa Cruz Biotechnology, Dallas, TX) or control scrambled siRNA was transfected.

MTS Assay

TNBCs (\(1 \times 10^4\) per well) were plated in equal numbers into 96-well plates, followed by the addition of MTS reagent (Abcam, Shanghai, China) as per the provided protocol. The cell proliferation rate was analyzed by measuring the absorbance at 490 nm.

CCK-8 Assay

In each well of a 96-well plate, \(1 \times 10^3\) cells were added, and 10 \(\mu\)L CCK-8 reagent (ApexBio, Houston, TX) in the fresh medium was replaced on days 1, 2, and 3, at the same time, and incubated for 1 hour at 37\(^\circ\)C and 5\% carbon dioxide. Absorbance was determined at 450 nm.

Colony Formation

Colony formation was performed as described in previous studies.\textsuperscript{19,20} The formation of visible colonies was assessed after seeding the cells (200 per well) into a fresh plate (six-well) and changing the medium every 2 days. Crystal violet staining of colonies was then performed after fixation with 4\% paraformaldehyde. The colonies were counted, and images were acquired.

Wound Healing Assay

To assess cell migration, a wound healing assay was conducted. After cells reached the appropriate confluence in plates (six-well), they were scraped in a straight line using...
the tip of a P-200 pipette, and floating cells were removed by washing thrice with phosphate-buffered saline. At 0 and 24 hours after scraping, six incidental images were acquired by using an inverted microscope. The wound surface areas were quantified by using ImageJ software (NIH, Bethesda, MD; http://imagej.nih.gov/ij).

Figure 1 Overexpression of ZNF33A relative to worse progression in triple-negative breast cancer (TNBC). A: Immunohistochemical (IHC) staining of ZNF33A in TNBC samples and adjacent nontumor tissues. B: mRNA level of ZNF33A in TNBC samples and adjacent nontumor tissues. C: Western blot analysis of ZNF33A in TNBC samples and adjacent nontumor tissues. D: Western blot analysis of ZNF33A in indicated TNBC cell lines. E: Relative level of ZNF33A in indicated TNBC cell lines was analyzed by real-time PCR. F: The grade of TNBC patients with different expression levels of ZNF33A. G: Overall survival time of patients with TNBC and different expression levels of ZNF33A. Data are expressed as means ± SD (A, B, and F). *P < 0.05; **P < 0.01; ***P < 0.001. Scale bar = 25 μm (A). N, normal; T, tumor.
Figure 2  ZNF33A regulates proliferation, growth, invasion, and migration in triple-negative breast cancer. A: ZNF33A expression was confirmed by Western blot analysis in the indicated cell lines. B: Cell proliferation was determined by MTS in the indicated cell lines. C: ZNF33A expression was confirmed by Western blot analysis in the indicated cell lines. D: Cell proliferation was determined by MTS in the indicated cell lines. E: Cell migration was analyzed by wound healing assay in the indicated cell lines. F: Cell invasion was analyzed by Transwell assay in the indicated cell lines. G: Cell migration was analyzed by wound healing assay in the indicated cell lines. H: Cell invasion was analyzed by Transwell assay in the indicated cell lines. Data are expressed as means ± SD (B-H). **P < 0.01. WT, wild type.
Transwell Invasion Assay

Precoating of Transwells (8 μm pores; Corning, Corning, NY) was performed with Matrigel (1:7 dilution in Dulbecco’s modified Eagle’s medium; Corning). In the upper chamber, 5 × 10^4 cells per well were seeded in serum-free medium after overnight starvation. These cells were left to enter the lower chamber, which contained culture medium with 10% fetal bovine serum. The invaded cells were stained with 0.5% crystal violet for 15 minutes after fixation with paraformaldehyde (4%). Images of migrated cells were acquired in five arbitrary fields using an inverted microscope, and cells were manually counted.

Real-Time RT-PCR

The real-time PCR assay was performed as described in previous studies. Total RNA was extracted from cell lines using TRIpure reagent (BioTek, Winooski, VT), and cDNA was synthesized by reverse transcription using HiScript Q RT

Figure 3 ZNF33A regulates tumor growth in vivo. A: Tumor volume curves of wild-type (WT) and ZNF33A-KO BT-594 xenograft. B: Tumor weight of WT and ZNF33A-KO BT-594 xenograft. C: Tumor volume curves of WT and ZNF33A-KO MDA-MB-231 xenograft. D: Tumor weight of WT and ZNF33A-KO MDA-MB-231 xenograft. E: Western blotting of ZNF33A in indicated tumors. F: Immunohistochemical staining of Ki-67 was analyzed in indicated tumors. G: Tumor volume curves of control and ZNF33A overexpressing BT-594 xenograft. H: Tumor weight of control and ZNF33A overexpressing BT-594 xenograft. I: Tumor volume curves of control and ZNF33A overexpressing MDA-MB-231 xenograft. J: Tumor weight of control and ZNF33A overexpressing MDA-MB-231 xenograft. K: Western blot analysis of ZNF33A in indicated tumors. L: Immunohistochemical staining of Ki-67 was analyzed in indicated tumors. Data are expressed as means ± SD (B, D, H, and J). *P < 0.05; **P < 0.01 (Wilcoxon test). Scale bars: 25 μm (F and L).
SuperMix provided in the real-time quantitative PCR kit (Vazyme, Nanjing, Jiangsu, China). Then, specific primers were used for RT-PCR with SYBR Green Master Mix (Bio-Rad, Hercules, CA) on an Applied Biosystems QuantStudio 3 Real-Time PCR System from Thermo Fisher Scientific (Waltham, MA). Accordingly, β-actin values were used for the normalization of target gene expression. The primers are listed as follows: ZNF33A, forward: 5'-TTCTCGGTGTTCCATACTGG-3', reverse: 5'-TGAAGACTAGACAGTGCTCTT-3'; c-Myc, forward: 5'-TGAGGACACGCCACAC-3', reverse: 5'-CAACATGTTTCTCTCTCTCTCTT-3'; and β-actin, forward: 5'-GCAACAGAGAGATGACAC-3', reverse: 5'-GTAACACATCAAGAGTCCA-3'.

Western Blot Analysis

Western blot analysis was performed as described in previous studies.21,23 After harvest, patient samples or cells were lysed on ice in radioimmunoprecipitation assay buffer. The protein samples were then analyzed by SDS-PAGE (10%) and transferred onto a polyvinylidene fluoride membrane. Blocking of membranes was performed in nonfat milk (5%) for 1 hour at room temperature, followed by three washes in tris-buffered saline with Tween 20 and overnight incubation with the primary antibody at 4°C. The membranes were incubated with secondary antibodies conjugated with horseradish peroxidase from MilliporeSigma at room temperature after washing thrice with tris-buffered saline with Tween 20 for 1 hour. Band detection was performed by using ECL Western Blotting Substrate according to the provided protocol. The primary antibodies used in this study were as follows: ZNF33A (AV51610; MilliporeSigma), β-actin (A5441; MilliporeSigma), and c-Myc (AB5232; MilliporeSigma).

Apoptosis Analysis

To estimate apoptosis, cells with condensed and fragmented nuclei were counted after staining of their nucleus with Hoechst 33258 (Invitrogen) as in previous studies.24–26 A minimum of 300 cells from each group were examined.

Xenograft

The animal experiment was approved by The First Affiliated Hospital of China Medical University and conducted according to the Guidelines for the Care and Use of Animals for Scientific Research. Four- to five-week-old BALB/c nude mice (18-20 g) were procured from Vital River (Beijing, China). Subcutaneous inoculation of wild-type and ZNF33A-KO MDA-MB-231 cells was performed on the back left sides of mice. After a tumor volume of approximately 100 mm³ was reached, mice were treated with either I-BET151 (30 mg/kg) or dimethyl sulfoxide by i.p. injection twice per week. Xenograft length and width were measured by using a Vernier caliper, and their volumes were calculated as (length × width²)/2. Twenty-one days after subcutaneous implantation, all mice were euthanized, and all xenografts were excised and weighed.

Immunohistochemical Analyses

The nude mouse xenografts were paraffin embedded, and sections of tissues were made for immunostaining of Ki-67 (#62548; Cell Signaling Technology, Danvers, MA) and cleaved caspase-3 (#9661; Cell Signaling Technology). The immunohistochemical score was the product of the positive tumor cell proportion and the staining intensity score. The intensity of staining was graded as follows: 1 = weak staining at 100× magnification and little or no staining at 40× magnification; 2 = medium staining at 40× magnification; and 3 = strong staining at 40× magnification. In each case, the intensity of immunostaining was scored and the proportion of positively stained tumor cells among all cells was estimated independently by two experienced pathologists who were blinded to the assay.

Statistical Analysis

Data are expressed as means ± SDs. Calculation of between-group comparisons was determined by one- or two-way analysis of variance using GraphPad Prism software 6 (GraphPad Software, San Diego, CA), and P < 0.05 was deemed statistically significant.

Results

ZNF33A Is Up-regulated in TNBC and Related to Poor Prognosis

To investigate the function of ZNF33A in TNBC, ZNF33A expression was analyzed in patient tissue samples from 62 pairs of TNBC using immunohistochemical staining, Western blot, and real-time PCR assays. The findings indicate that ZNF33A was highly expressed in TNBC tissues compared with adjacent healthy breast tissues (Figure 1A-C). Moreover, ZNF33A levels were analyzed in TNBC cell lines. As shown in Figure 1D and E, the levels of ZNF33A in the cell lines of human healthy breast epithelial cell lines HBL-100 and MCF-10 A were much lower than that in human TNBC cells. These observations indicate the aberrant expression of ZNF33A in TNBC. Furthermore, a high level of ZNF33A expression in TNBC patient specimens was associated with high grade and a shorter duration of survival (Figure 1F and G). Thus, these data show that ZNF33A overexpression may be a biomarker for TNBC prognosis.
ZNF33A Overexpression Increases the Growth and Invasive and Migratory Properties of TNBC Cells

Considering the clinical significance of ZNF33A in TNBC, we next examined the role of ZNF33A in TNBC cell behavior. To this end, the CRISPR/cas9 system was used to knock out ZNF33A in TNBC cells (Figure 2A), and cell growth ability was then determined through MTS, CCK-8 cell proliferation, and colony formation assays. The results indicated that ZNF33A inhibition in vitro markedly slowed the proliferation of TNBC cells (Figure 2Ba and Supplemental Figure S1A-F). In contrast, enhanced expression of ZNF33A through ectopic transfection of ZNF33A plasmid increased the proliferation of TNBC cells (Figure 2C-D and Supplemental Figure S1G-H). The effect of ZNF33A on cell invasion and migration was also

Figure 4  ZNF33A regulates bromodomain and extraterminal domain inhibitor (BET) inhibitor sensitivity in triple-negative breast cancer (TNBC). A: MDA-MB-231 cells with ZNF33A knockout or ZNF33A overexpression were treated with different types of inhibitors for 72 hours. The 50% inhibitory concentration (IC50) was analyzed and IC50 ratios were determined and are shown in a heatmap. B: BT-594 cells with ZNF33A knockout or ZNF33A overexpression were treated with different types of inhibitors for 72 hours. The IC50 was analyzed and IC50 ratios were determined and are shown in a heatmap. C: Control or ZNF33A overexpressing MDA-MB-231 or BT-594 cells were treated with increasing concentrations of I-BET151 for 72 hours. Growth inhibition was analyzed by MTS. D: Control or ZNF33A overexpressing MDA-MB-231 or BT-594 cells were treated with 5 μM I-BET151 for 24 hours. Cell growth was determined by colony formation. E: WT or ZNF33A-KO MDA-MB-231 or BT-594 cells were treated with increasing concentrations of I-BET151 for 72 hours. Growth inhibition was analyzed by MTS. F: WT or ZNF33A-KO MDA-MB-231 or BT-594 cells were treated with 1 μM I-BET151 for 24 hours. Cell growth was determined by colony formation. G: WT or ZNF33A-KO MDA-MB-231 or BT-594 cells were treated with 1 μM I-BET151 for 24 hours. Apoptosis was analyzed by counting cells with condensed and fragmented nuclei after nuclear staining with Hoechst 33258. H: WT, ZNF33A-KO, or ZNF33A-KO with ZNF33A overexpressing MDA-MB-231 or BT-594 cells were treated with increasing concentrations of I-BET151 for 72 hours. Growth inhibition was analyzed by MTS. Data are expressed as means ± SD (G). ***P < 0.001, 5-FU, 5-fluorouracil.
analyzed. The data show that depletion of ZNF33A markedly suppressed cell invasion and migration (Figure 2E-F and Supplemental Figure S2A-D), whereas overexpression of ZNF33A increased cell invasion and migration in TNBC cells (Figure 2G-H and Supplemental Figure S2E-F). Thus, ZNF33A was found to regulate the invasion, growth, and migration of TNBC cells.

**ZNF33A Increases TNBC Tumor Growth in Vivo**

Next, the in vivo tumor progression of cancer cells was assessed in a xenograft tumor mouse model. ZNF33A knockout markedly suppressed the growth of TNBC cells (Figure 3A-E) and reduced the number of cells positive for Ki-67 in the tumors (Figure 3F). In contrast, increased expression of ZNF33A by ectopic transfection improved the proliferative capacity of TNBC tumors (Figure 3G-3L). Thus, these data indicate that ZNF33A promotes TNBC tumor growth in vivo.

**ZNF33A Knockout Enhances the Sensitivity of TNBC Cells to BET Inhibitors**

The role of ZNF33A was explored by evaluating the sensitivity toward small molecular drugs after ZNF33A knockout or high expression in MDA-MB-231 cells using a series of molecules (Figure 4A). The drug sensitivity of these molecules was estimated by determining the 50% inhibitory concentration values of every group. Then, in the ZNF33A knockout/overexpressed group, the 50% inhibitory concentration values of these inhibitors were normalized to the 50% inhibitory concentration values of the control group; they are shown as a heatmap in Figure 4A. MDA-MB-231 cells were found to be sensitive to BET inhibitors after ZNF33A knockout (Figure 4A), and increased levels of ZNF33A led to enhanced resistance of MDA-MB-231 cells to BET inhibitors (Figure 4A). These results were also observed in BT-594 cells (Figure 4B). Therefore, these results indicate that ZNF33A regulates BET inhibitor sensitivity in TNBC.

Next, overexpression of ZNF33A significantly increased in Q12 the 50% inhibitory concentration values of I-BET151 by greater than fourfold in MDA-MB-231 and BT-594 cells (Figure 4C). Furthermore, BET inhibitor resistance due to ZNF33A was also confirmed by a colony formation assay, wherein the inhibitory effects of I-BET151 on the viability of MDA-MB-231 cells decreased markedly (Figure 4D), and the outcomes were consistent in BT-594 cells (Figure 4D). We then examined whether the depletion of ZNF33A could sensitize cancer cells to BET inhibition. Indeed, cancer cell growth was more suppressed in the ZNF33A-KO group due to BET inhibitors than in the control group, as revealed by MTS and colony formation assays (Figure 4E and F). In contrast, ZNF33A repression increased cancer cell apoptosis after BET inhibitor treatment (Figure 4G). In addition, restoring the expression of ZNF33A in the ZNF33A-KO group led to MDA-MB-231 cell resistance to I-BET151 (Figure 4H). Thus, the role of ZNF33A in the regulation of BET inhibitor sensitivity needs further exploration.
Figure 6  c-Myc is critical for ZNF33A-mediated bromodomain and extraterminal domain inhibitor (BET) inhibitor resistance. A: Western blot analysis of c-Myc in ZNF33A knockdown cells. B: mRNA level of c-Myc in ZNF33A knockdown cells. C: Western blot analysis of c-Myc in wild-type (WT) and ZNF33A-KO cells. D: mRNA level of c-Myc in WT and ZNF33A-KO cells. E: Western blot analysis of c-Myc in ZNF33A overexpressing cells. F: mRNA level of c-Myc in ZNF33A overexpressing cells. G: The correlation of c-Myc and ZNF33A was analyzed (Spearman $r = 0.8072$, $P < 0.001$). H: WT, ZNF33A-KO, or ZNF33A-KO with c-Myc overexpressing MDA-MB-231 or BT-594 cells were treated with increasing concentrations of I-BET151 for 72 hours. Growth inhibition was analyzed by MTS. I: Control, ZNF33A overexpressing, or ZNF33A overexpressing with c-Myc knockdown MDA-MB-231 or BT-594 cells were treated with increasing concentrations of I-BET151 for 72 hours. Growth inhibition was analyzed by MTS. Data are expressed as means ± SD (B, D, and F). ***$P < 0.001$. IC$_{50}$, 50% inhibitory concentration.
ZNF33A is crucial in conferring resistance to BET inhibitors.

Depletion of ZNF33A Enhances the Sensitivity of TNBC Cells to BET Inhibitors in Vivo

Next, in vivo assessment of ZNF33A-mediated chemosensitization by BET inhibitors was performed in nude mice by treating wild-type and ZNF33A-KO MDA-MB-231 xenograft tumors with I-BET151. Indeed, tumor growth was slower (Figure 5A–C), and more apoptotic cells were observed in the ZNF33A-KO group treated with I-BET151 compared with the control group (Figure 5D). Therefore, these data indicate that silencing ZNF33A mediated MDA-MB-231 cell sensitivity to BET inhibitors in TNBC.

ZNF33A Enhances c-Myc Expression in TNBC Cells

Several factors mediate the resistance of cancer cells to BET inhibitors; c-Myc is one such factor that has a crucial role in TNBC cells. Hence, the capacity of ZNF33A in TNBC cells to regulate c-Myc expression was examined. Two different siRNAs were used to silence ZNF33A expression and assess any change in c-Myc expression (Figure 6A and B). The mRNA and protein levels of c-Myc were decreased in TNBC cells with ZNF33A knockdown (Figure 6A and B). The mRNA and protein levels of c-Myc also decreased in ZNF33A-KO cells (Figure 6C and D). In contrast, overexpression of ZNF33A led to up-regulation of c-Myc in MDA-MB-231 and BT-594 cells (Figure 6E and F). Moreover, a positive correlation between ZNF33A mRNA and c-Myc was observed in patients with TNBC (Figure 6G). In addition, overexpression of c-Myc in ZNF33A-KO cell lines attenuated ZNF33A depletion-mediated I-BET151 sensitivity in TNBC (Figure 6H).

Next, c-Myc was knocked down in ZNF33A overexpressing TNBC cells, and we found that c-Myc knockdown resensitized ZNF33A overexpressing TNBC cells to I-BET151 (Figure 6I). Taken together, these findings suggest that c-Myc may play a key role in ZNF33A-mediated BET inhibitor resistance.

Discussion

Targeted therapy for TNBC, a highly aggressive cancer, is still lacking, making it important to identify efficient targets for its treatment.5 The biological role and clinical characteristics of ZNF33A in TNBC were investigated. These data show the potential of ZNF33A as a biomarker for TNBC prognosis, as well as its role in enhanced in vitro and in vivo tumor growth, indicating it as a possible target for TNBC treatment. Importantly, ZNF33A played a crucial role in the sensitivity of TNBC cells to BET inhibitors (Supplemental Figure S3).

Clinical trials have assessed BET inhibitors and have shown their single-agent efficacy against NUT carcinomas and some hematologic malignancies.29 The interaction of BET inhibitors with the bromodomain of BRD4 is competitive in nature and leads to displacement of the oncogenic protein bound with BRD, resulting in inhibition of tumor cell growth.30 This antitumor effect of BET inhibitors can potentially be used for the treatment of cancer. However, the resistance of cancer cells to BET inhibitors impedes their clinical application.31 Therefore, to develop precision and personalized therapies, it is essential to predict BET inhibitor sensitivity using biomarkers. In addition to SPOP (sporcle-type pox virus and zinc finger protein) mutations, colorectal cancer cells with a CIMP (CpG island methylator phenotype) are sensitive to c-Myc repression mediated by JQ1.32 In TNBC cells, BET inhibitor resistance characterizes bromodomain-independent activities and hyperphosphorylation of BRD4.33 Various groups have used preclinical models and described pleiotropic resistance mechanisms to BET inhibitor in reprogramming of the kinome in ovarian cancer, activation of compensatory β-catenin in leukemia, and conformational changes in BRD4 in TNBC.34 These findings indicate that the acquired BET inhibitor resistance mechanisms are specific to tumor types. Thus, identification of biochemical mechanisms causing acquired resistance to BET inhibitors suggests specific treatment modalities to resensitize prostate cancer cells to BET inhibitors and to design combination treatments for castration-resistant prostate cancer. In future study, the mode of regulation of c-Myc by ZNF33A will be examined.

c-Myc is a transcription factor that regulates the expression of various genes.35 It is among the most significant regulators in promoting TNBC carcinogenesis by modulating cell growth, metabolism, metastasis, and apoptosis.36 An understanding of the c-Myc–mediated regulatory mechanism will aid in designing novel treatment modalities for TNBC.37 c-Myc is regulated by various transcription factors, including BRD4, JunD, and APC.38 The expression of c-Myc in cancer cells increases due to aberrant activation of oncogenic pathways, such as the mitogen-activated protein kinase or phosphatidylinositol 3-kinase/protein kinase B pathways.39,40 ZNF33A is a transcriptional factor and should regulates many genes. In the current study, the results showed that ZNF33A mediated up-regulation of c-Myc transcript and protein in TNBC, which indicates that c-Myc should be a target of ZNF33A. The mechanisms by which ZNF33A regulates c-Myc will be investigated in the future. Furthermore, a reduced level of ZNF33A increases TNBC cell sensitivity to BET inhibitors. Remarkably, the level of c-Myc is deemed a crucial mediator for inhibitors of BET in TNBC.41 Thus, compared with single I-BET151 treatment, ZNF33A knockout along with I-BET151 treatment reduced the resistance of cancer cells to I-BET151 in MDA-MB-231 and BT-594 cells. Moreover, ZNF33A increased c-Myc production at the level of transcription in TNBC cells by interacting with BRD4. ZNF33A may act as an activator of BRD4 activity in TNBC cells.
Conclusions

These data show the potential of ZNF33A overexpression as a biomarker for TNBC patient prognosis. ZNF33A promoted TNBC growth in vitro and in vivo, and also contributed to their resistance to BET inhibitors by enhancing c-Myc levels in cancer cells. Thus, the tumor growth–promoting effect of ZNF33A in TNBC and the enhanced sensitivity of xenograft models to BET inhibitors due to ZNF33A knockout are presented.

Acknowledgments

Not applicable.

Author Contributions

X. Wang, X. Wei, and P. Xing conceived and designed the experiments; X. Wang, X. Wei, and Y.C. performed most of the experiments and data analysis; X. Wang and P.X. drafted the manuscript. All authors read and approved the final manuscript.

Supplemental Data

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