



## Research Article

# NEIL3 Regulates Paclitaxel Resistance Via P300-Mediated Modification of H3K27ac in TNBC

 Ya-Feng Zhang,<sup>1</sup>  Xiu-Mei Wang<sup>2</sup>

<sup>1</sup>Department of Thyroid and breast Surgery, The Affiliated Hospital of Inner Mongolia Medical University, Hohhot, China

<sup>2</sup>Medical Oncology, Affiliated Cancer Hospital of Inner Mongolia Medical University, Hohhot, Inner Mongolia, China

### Abstract

**Objectives:** To explore the function of NEIL3 in the Triple-negative breast cancer (TNBC) with PTX resistance. And the relationship between the NEIL3 and H3K27ac, and the underlying mechanisms were also detected.

**Methods:** The NEIL3 expression in the humane TNBC and cells with PTX resistance was determined by qPCR and western blot. The cell viability was detected by CCK-8. The NEIL3 was silenced or overexpressed to show its function on the cell viability, cell invasion, mitochondrial respiration (cellular oxygen consumption rates, OCR). The interaction between the NEIL3 and P300 was showed by Luciferase assay. And the chromatin immunoprecipitation assays were used to show the relationship between NEIL3 H3K27ac and P300.

**Results:** NEIL3 expression was low in the PTX resistance TNBC cancer tissue and MDA-MB-231, BT-20 cells. When the NEIL3 was silenced, the OCR, PTX resistance and invasion was promoted in MDA-MB-231 and BT-20 cells. While the NEIL3 overexpressed, the PTX resistance, invasion and OCR was inhibited. Silenced P300 can promote the interaction between the NEIL3 promoter and H3K27ac, leading to increase NEIL3 expression.

**Conclusions:** NEIL3 expression was regulated by P300 modifications of H3K27ac. P300 recruits to the promoter region of NEIL3 through acetylation of H3K27ac, synergistically inhibiting the transcription and expression of NEIL3, ultimately inducing the PTX resistance in TNBC.

**Keywords:** NEIL3, TNBC, P300, H3K27ac, PTX resistance

**Cite This Article:** Zhang YF, Wang XM. NEIL3 Regulates Paclitaxel Resistance Via P300-Mediated Modification of H3K27ac in TNBC. EJMO 2024;8(4):433–442.

Triple-negative breast cancer (TNBC) is one of the subtypes of breast cancer. It is characterized by a high degree of malignancy and a low recovery rate.<sup>[1,2]</sup> Clinically, chemotherapy has always been used to treat TNBC.<sup>[1,3,4]</sup> However, drug resistance is often observed with chemotherapeutics such as paclitaxel (PTX).<sup>[2]</sup> The mechanism of PTX anti-tumor action is largely reflected by its ability to promote microtubule protein aggregation, inhibit depolymerization, maintain microtubule protein stability, hinder cell mitosis, and cause cell arrest in G2 and M phases.<sup>[5,6]</sup> PTX

can effectively prevent the proliferation of cancer cells and plays an anti-cancer role.<sup>[7]</sup> Long-term treatment with PTX gradually leads to the development of tumor cells, leading to a weakened or ineffective anti-cancer effect of PTX.<sup>[6]</sup> The emergence of PTX resistance often limits its efficacy in the treatment of TNBC.<sup>[5,6]</sup> Furthermore, TNBC cells from different patients may have different mechanisms of drug resistance, thus complicating the treatment.<sup>[6,7]</sup> The mechanisms underlying Paclitaxel resistance in TNBC mainly contained: DNA damage repair is closely related to the mecha-

**Address for correspondence:** Xiu-Mei Wang, MD. Medical Oncology, Affiliated Cancer Hospital of Inner Mongolia Medical University, Hohhot, Inner Mongolia, China

**Phone:** +864713280928 **E-mail:** wangxiumei75@aliyun.com

**Submitted Date:** October 04, 2024 **Accepted Date:** November 10, 2024 **Available Online Date:** December 09, 2024

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nism of action of chemotherapy drugs and is considered a key mechanism of tumor chemoresistance.<sup>[8-10]</sup> Paclitaxel, docetaxel, platinum drugs, and other chemotherapy drugs can induce DNA damage in tumor cells. If the damaged DNA cannot be repaired, the tumor cells are killed by the chemotherapy drugs.<sup>[9,10]</sup> And Epigenetic modifications, represented by histone acetylation, are at the forefront of biomedical research in recent years.<sup>[11]</sup> It has been found that there is a close connection between epigenetics and the occurrence, development, and drug resistance mechanisms of tumors.<sup>[12]</sup>

Nei endonuclease VIII-like 3 (NEIL3) is a member of the FEN1 nuclease superfamily, which is implicated in DNA repair and replication.<sup>[13]</sup> NEIL3 is involved in the restart and stabilization of replication forks, as well as certain steps in DNA damage repair.<sup>[13,14]</sup> When DNA replication encounters obstacles, such as DNA damage or replication fork arrest, NEIL3 is involved in the DNA repair in the form of cleavage activity, thereby maintaining genome stability.<sup>[15]</sup>

Acetylated H3K27 (H3K27ac) is a form of histone modification that occurs on the 27<sup>th</sup> lysine residue of histone H3.<sup>[16,17]</sup> This modification is an important mechanism for regulating intracellular gene expression, involving several biological processes such as cell proliferation, differentiation, apoptosis, and the occurrence and development of diseases.<sup>[18-20]</sup> Acetylation of H3K27 may alter the structure of chromatin, making DNA more easily accessible to transcription factors and other regulatory proteins, thereby promoting gene expression.<sup>[21,22]</sup> H3K27ac can play a crucial role in cellular response to environmental stimuli, developmental processes, and disease states.<sup>[23,24]</sup> In addition, individual differences, gene mutations, drug metabolism, and other factors may also affect the drug resistance of PTX in TNBC.<sup>[5,6]</sup> TNBC is characterized by DNA damage. NEIL3 has been reported to be involved in the restart and stabilization functions.<sup>[13]</sup> H3K27ac is promoted in cancers and inhibiting H3K27ac decreases tumor formation.<sup>[25]</sup> High H3K27ac levels were associated with poor prognosis in liver cancer patients.<sup>[16]</sup> In this study, the function of NEIL3 in TNBC with PTX resistance was explored. The relationship between NEIL3 and H3K27ac, and the underlying mechanisms were investigated.

## Methods

### Patient Sample Collection

For this study, 30 samples of TNBC cancer tissue and 30 samples of TNBC paracancerous tissue were collected. The inclusion criteria were as follows: patients with breast cancer type TNBC as the primary tumour receiving PTX treatment. The exclusion criteria were as follows: patients with

other cancer metastases. The TNBC cancerous and TNBC paracancerous samples, we selected tissue ~3 cm away from the TNBC cancerous lesions as the paracancerous tissue. Additionally, institutional protocols, approved by the Institutional Ethical Review Board of Inner Mongolia Medical University (NO. YDK2020021025), were followed.

### Cell Culture

MDA-MB-231 and BT-20 cell lines were cultured in the Dulbecco modified Eagle's medium (DMEM) (Sigma-Aldrich) with 10% fetal bovine serum (FBS, Gibco) as well as 0.1 mg/mL streptomycin and 100 U/mL penicillin. Cells were cultured at 37° in 5% CO<sub>2</sub>. To establish PTX cell lines, the concentration of PTX (Sigma-Aldrich, USA) in the culture medium was increased gradually from 1 nM to 100 nM for 6 months. The culture medium with PTX was changed every 2 days.<sup>[26]</sup>

### Cell Counting

Cell proliferation was evaluated using this kit: CCK-8 (K1018; Apexbio, Houston, TX, USA). Cells were plated in 96-well plates (1 × 10<sup>4</sup> cells per well, 100 µL/well), and different drug concentrations and 10 µL of solution (CCK-8) were added at the following time points: 24 h, 48 h, and 72 h. The plates were kept at 37°C. Absorbance values at 450 nm were measured using an enzyme marker.

### IC50 Assay

The MTS assay (Promega, Beijing, China) was used to determine the IC50s of PTX. Briefly, cells were seeded in 96-well plates with gradated concentrations of the PTX, and then the 96-well plates were incubated at 37° for 48 h. The absorbance at 492 nm was measured by using a microplate reader (TECAN Spark 10 M, Shanghai, China). A total of 1000 cells/well were seeded in 6-well plates. The experimental data are transformed into percentage inhibition rates. For example, if the cell growth rate of the control group is 100%, and the cell growth rate at a certain concentration is 50%, then the inhibition rate is 50%.

### Real-Time qPCR Analysis

Total RNA was extracted from cells using the TRIzol reagent (Invitrogen, USA). RNA was reverse transcribed into complementary DNA (cDNA) using a TIANScript RT kit (Tiangen Biotech, China). A SYBR Premix Ex Taq TM II Kit (Takara, Japan) was used to identify relative gene expression. The primers were as follows: NEIL3 forward primer: GCAGTAAACACAACCGCCTC, reverse primer: AAGGACAAATCTGCCCATTCAA; GAPDH forward primer: CAATGCCTCTGCACCACCAACTGC, reverse primer: GCAGTTGTGGTGCAGGAGGCATTG.

## Western Blotting

Total proteins were first extracted and subsequently, the concentration of each sample was determined using a bicinchoninic acid kit (23227, Thermo Fisher Scientific). Protein separation was achieved with polyacrylamide gel electrophoresis. Proteins were next transferred to membranes (polyvinylidene fluoride; Millipore, Billerica, MA, USA), followed by blocking with 5% bovine serum albumin (BSA) for 1 h at room temperature. The membranes were incubated overnight with primary antibodies at 4°C. Next, the membranes were incubated with dilutions of goat anti-rabbit immunoglobulin G (ab205718, Abcam) at room temperature for 1.5 h. After the incubation period, a developing liquid (NCI4106; Pierce, Rockford, IL, USA) was used to develop the membranes. Clinx Chemi Analysis (ChemiScope 6000, Shanghai, China) software was used for protein quantification.<sup>[26]</sup>

## Cell Invasion Assay

The cells were seeded in 6-well plates. Subsequent operations were performed when the cell confluency was greater than 90%. The cells were drawn in a straight line. The floating cells were washed with phosphate-buffered saline (PBS). The cell migration was observed and recorded for 0 h under an inverted phase contrast microscope, and subsequently, a 6-well plate was placed in the cell culture incubator for 2 h. The migration of each group of cells was recorded with an inverted microscope.

## Extracellular Flux (XF) Analysis

The cells were cultured in XF-24 plates for 24 h at 37° and 5% CO<sub>2</sub> (Agilent, Santa Clara, CA). Next, cells were cultured without CO<sub>2</sub> for 1 h. Oligomycin (an ATP synthase inhibitor) was added to the seahorse gauging plate labeled as "A well." The carbonyl cyanide p-trifluoromethoxy (FCCP; uncoupler) was added and labeled as "B well." The labeled C well to which a mixture of antimycin A and rotenone a mixture of antimycin A with 1:1 by Seahorse XF24 Extracellular Flux Analyzer. The cellular oxygen consumption rates (OCR) were obtained.

## Cell Transfection

The siRNA and overexpression plasmid of NEIL3 were obtained from GenePharma (Shanghai, China). Lipofectamine 3000 was used to transfect plasmids or oligonucleotides into cells according to the protocols. The sequence of S1 GCAGGACUUGCUCUCUCUATT UAGAGAGAGCAAGUCCUGCTT, S2 GCUCACCAAAGAUUUGAUUTT AAUCAAUCUUUGGUGAGCTT, NC UUCUCCGAACGUGUCACGUTT ACGUGACACGUUCGGAGAATT.

## Luciferase Assay

The pRL-TK vector and pGL3-basic plasmid containing the NEIL3 promoter were transfected into cells with 10 μM C646 (a P300 inhibitor for histone acetyltransferase) via Lipofectamine 300. The luciferase activity was recorded.

## Chromatin Immunoprecipitation Assay

The cells were fixed and probed with an antibody (anti-H3K27ac, anti-p300, or IgG antibodies). The NEIL3 promoter binding with H3K27ac or p300 was determined by PCR. The sense primer was 5'-CCCTTGGCTCATTCCG-3' and the antisense primer was 5'-TCCTTCGCCTTCCTTCC-3'.

## Statistical Analysis

GraphPad Prism software (La Jolla, CA, USA) was used to compare the groups. The independent sample t-test was used for comparisons between the groups. The statistical model included replicates, and statistical significance was set at a 95% confidence level ( $p < 0.05$ ). All data are presented as means ± standard deviation (SD).

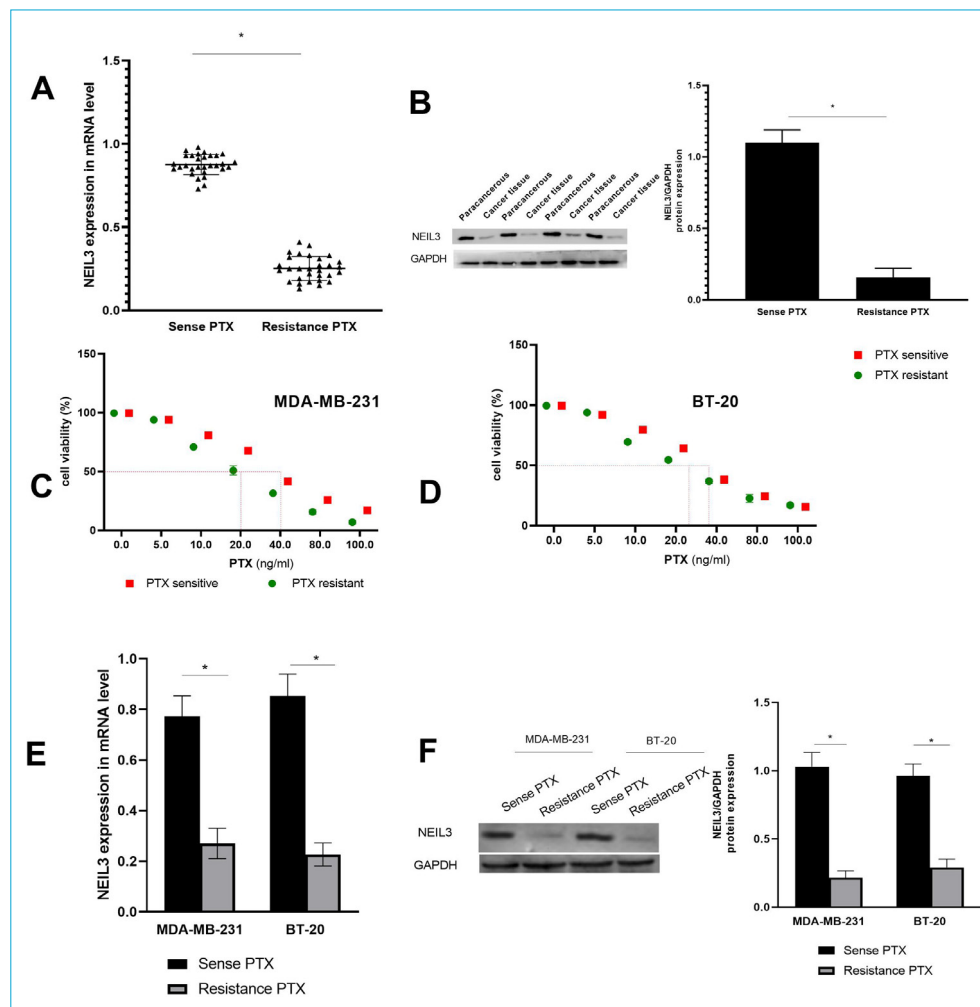
## Results

### NEIL3 Low Expression in PTX Resistance Tissue and Cells

We collected 30 TNBC PTX-resistant cancer tissue cases and 30 TNBC PTX sense cancer tissue cases to study the expression of NEIL3. NEIL3 expression was lower ( $p < 0.05$ ) (Fig. 1a and b) in PTX-resistant cancer tissue. PTX-resistant cells were established in MDA-MB-231 and BT-20 cells (Fig. 1c and d). The IC<sub>50</sub> of MDA-MB-231 and BT-20 cells in PTX sense was 20ng/ml and 20ng/ml respectively. And the IC<sub>50</sub> of MDA-MB-231 and BT-20 cells in PTX resistant was 40ng/ml and 38.7ng/ml respectively. The expression of NEIL3 in MDA-MB-231 and BT-20 cells was higher than the PTX resistance in MDA-MB-231 and BT-20 cells ( $p < 0.05$ ) (Fig. 1e and f). These results demonstrated that NEIL3 expression was low in PTX-resistant TNBC cancer tissue and MDA-MB-231 and BT-20 cells. Hence, NEIL3 may contribute to PTX resistance in TNBC.

### NEIL3 Silences Accelerates PTX Resistance in MDA-MB-231 and BT-20 Cells

We next investigated the contribution of NEIL3 to PTX resistance in MDA-MB-231 and BT-20 cells. The siRNA-mediated silencing of NEIL3 showed that its expression was lower in si1 and si2 groups ( $p < 0.05$ ) (Fig. 2a-d and Sup. Fig. 2b). In MDA-MB-231 cells, the IC<sub>50</sub> value in NC, si1 and si2 groups



**Figure 1.** NEIL3 expression in TNBC tissue and TNBC cells. A. NEIL3 mRNA expression in sense PTX and resistant PTX tissue. B. NEIL3 protein expression in sense PTX and resistant PTX tissue. C&D. The MTS assay was conducted to determine the IC<sub>50</sub> of PTX in MDA-MB-231 and BT-20 cells. E. NEIL3 mRNA expression in sense PTX and PTX-resistant MDA-MB-231 and BT-20 cells. F NEIL3 protein expression in sense PTX and PTX-resistant MDA-MB-231 and BT-20 cells. \*Data are presented as mean±standard deviation (SD) (\*p<0.05). The independent sample t-test was employed for making comparisons between the groups. Each experiment was replicated thrice. The sense PTX group was the control.

was 20 ng/mL, 38.5 ng/mL, and 40 ng/mL, respectively. The IC<sub>50</sub> value in BT-20 cells was 24.5 ng/mL, 38.7 ng/mL, and 28.7 ng/mL in NC, si1 and si2 groups, respectively. The IC<sub>50</sub> value in the si1 and si2 groups was higher than that in the NC groups in both cells (p<0.05) (Fig. 2e-j).

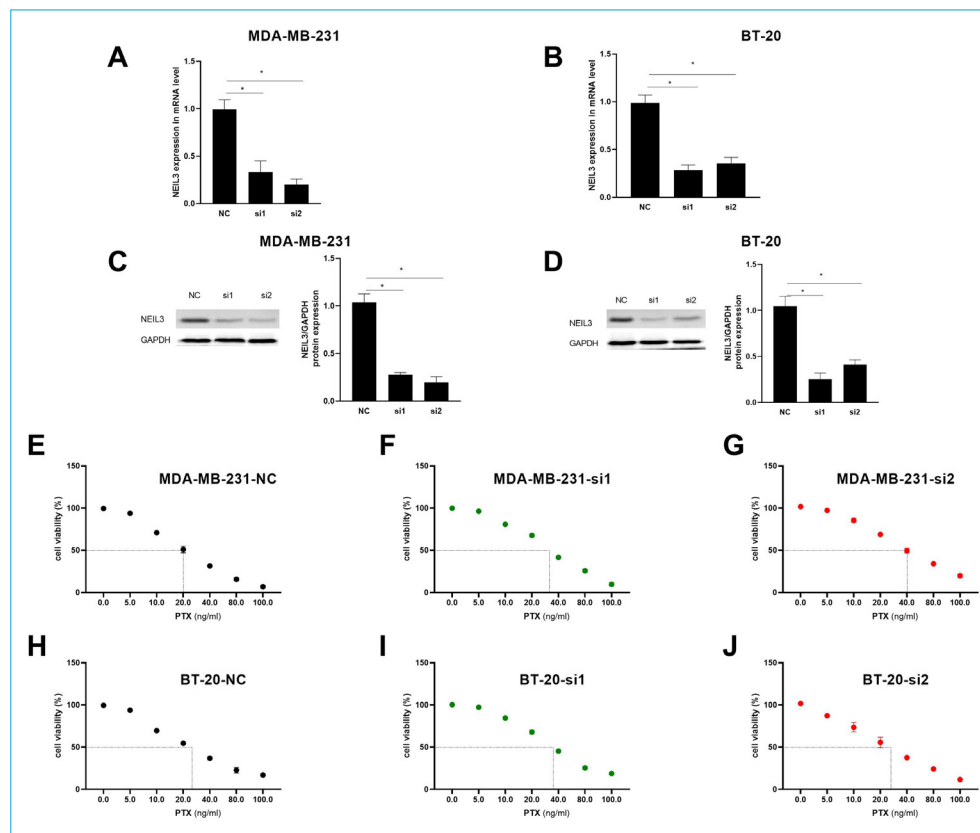
### NEIL3 Silencing Promotes the Invasion of MDA-MB-231 and BT-20 Cells with PTX Resistance

We conducted the cell scratch assay to determine the changes in the invasion in MDA-MB-231 and BT-20 cells with PTX resistance following NEIL3 silencing. The migration distance in the si1 and si2 groups was lower than that in the NC group in MDA-MB-231 and BT-20 cells with PTX

resistance (p<0.05) (Fig. 3). This implies that NEIL3 inhibited the invasion of MDA-MB-231 and BT-20 cells with PTX resistance.

### NEIL3 Silencing Promotes OCR Functions in MDA-MB-231 and BT-20 Cells with PTX Resistance

Next, we detected OCR in MDA-MB-231 and BT-20 cells with PTX resistance following NEIL3 silencing to study the influence of NEIL3 on mitochondrial respiration functions. The basal respiration, ATP-linked respiration, and maximal respiration in the si1 and si2 groups were significantly higher than those in the NC group (p<0.05) (Fig. 4). These results imply that OCR functions are regulated by NEIL3.



**Figure 2.** Inhibited NEIL3 expression facilitates PTX resistance in MDA-MB-231 and BT-20 cells. A-D qPCR and western blotting detected NEIL3 expression in NEIL3-silenced MDA-MB-231 and BT-20 cells. E-J. The MTS assay was conducted to determine the  $IC_{50}$  of PTX in MDA-MB-231 and BT-20 cells after treatment. The NC group was the transfer the sequence which was no influence on the cells.

### NEIL3 Overexpression Inhibits Cell Proliferation, Invasion, and Mitochondrial Respiration in MDA-MB-231 and BT-20 Cells with PTX Resistance

We further explored the functions of NEIL3, for which we overexpressed NEIL3 in MDA-MB-231 and BT-20 cells with PTX resistance (Sup. Fig. 1). The  $IC_{50}$  of MDA-MB-231 and BT-20 cells (5.0 ng/mL and 4.57 ng/mL, respectively) was lower than that of the NC group ( $p < 0.05$ ) (Fig. 5a and b). The migration distance in the NEIL3 overexpression groups was no different from the NC group in MDA-MB-231 and BT-20 cells with PTX resistance ( $p > 0.05$ ) (Fig. 5c-f). Basal respiration, ATP-linked respiration, and maximal respiration in the overexpression group was lower than that in the vector group in MDA-MB-231 and BT-20 cells with PTX resistance ( $p < 0.05$ ) (Fig. 5g-j).

### P300 Modifications of H3K27ac Regulates NEIL3

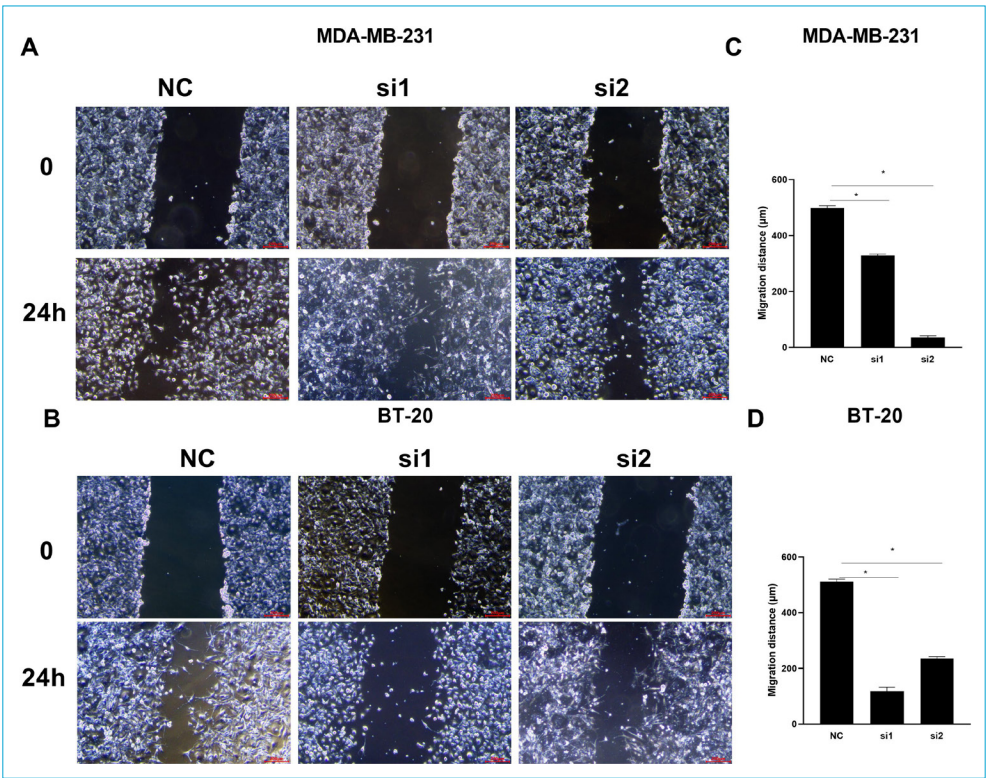
We next elucidated the mechanism by which NEIL3 contributed to PTX resistance in TNBC. The pRL-TK vector or pGL3-basic plasmid with NEIL3 promoter sequences or the NEIL3 mutant promoter sequences (Fig. 6a) was transfected into PTX-resistant MDA-MB-231 cells with or without

C646 (P300 inhibitor). The NEIL3 promoter was activated by treating MDA-MB-231 cells with PTX resistance with C646 ( $p < 0.05$ ) (Fig. 6b). However, no significant difference was noted between C646 and vehicle groups when the NEIL3 promoter was mutated. The expression of NEIL3 was increased and H3K27ac was inhibited by C646 (Fig. 6c and d). The NEIL3 promoter increased the acetylation of H3K27 (Fig. 6e) and P300 (Fig. 6f) in MDA-MB-231 cells with PTX resistance. The interaction between H3K27ac and P300 with NEIL3 promoter in MDA-MB-231 cells with PTX resistance was inhibited when P300 was silenced (Fig. 6g). These findings suggest that NEIL3 was regulated by P300-mediated modifications of H3K27ac. Hence, P300 is recruited to the promoter region of NEIL3 through the acetylation of H3K27ac, thereby synergistically inhibiting the transcription and expression of NEIL3, and ultimately inducing PTX resistance in TNBC.

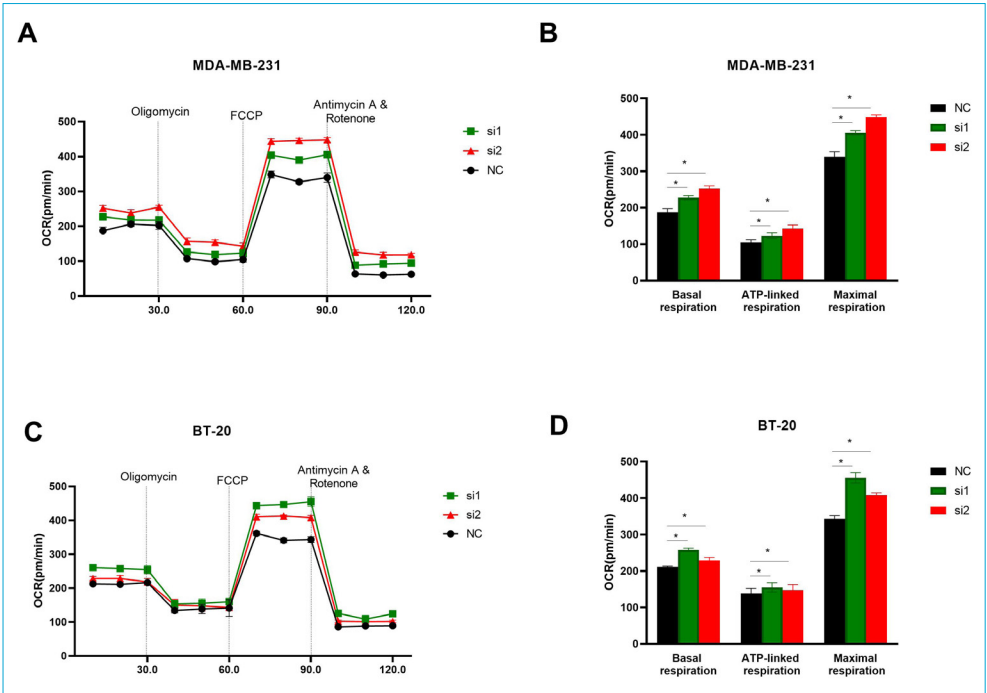
### Discussion

TNBC accounts for 15 to 20% of all breast cancer types.<sup>[27,28]</sup> PTX is the mainstay drug for treating TNBC. However, PTX resistance in patients often occurs.<sup>[28-30]</sup> In this study, we

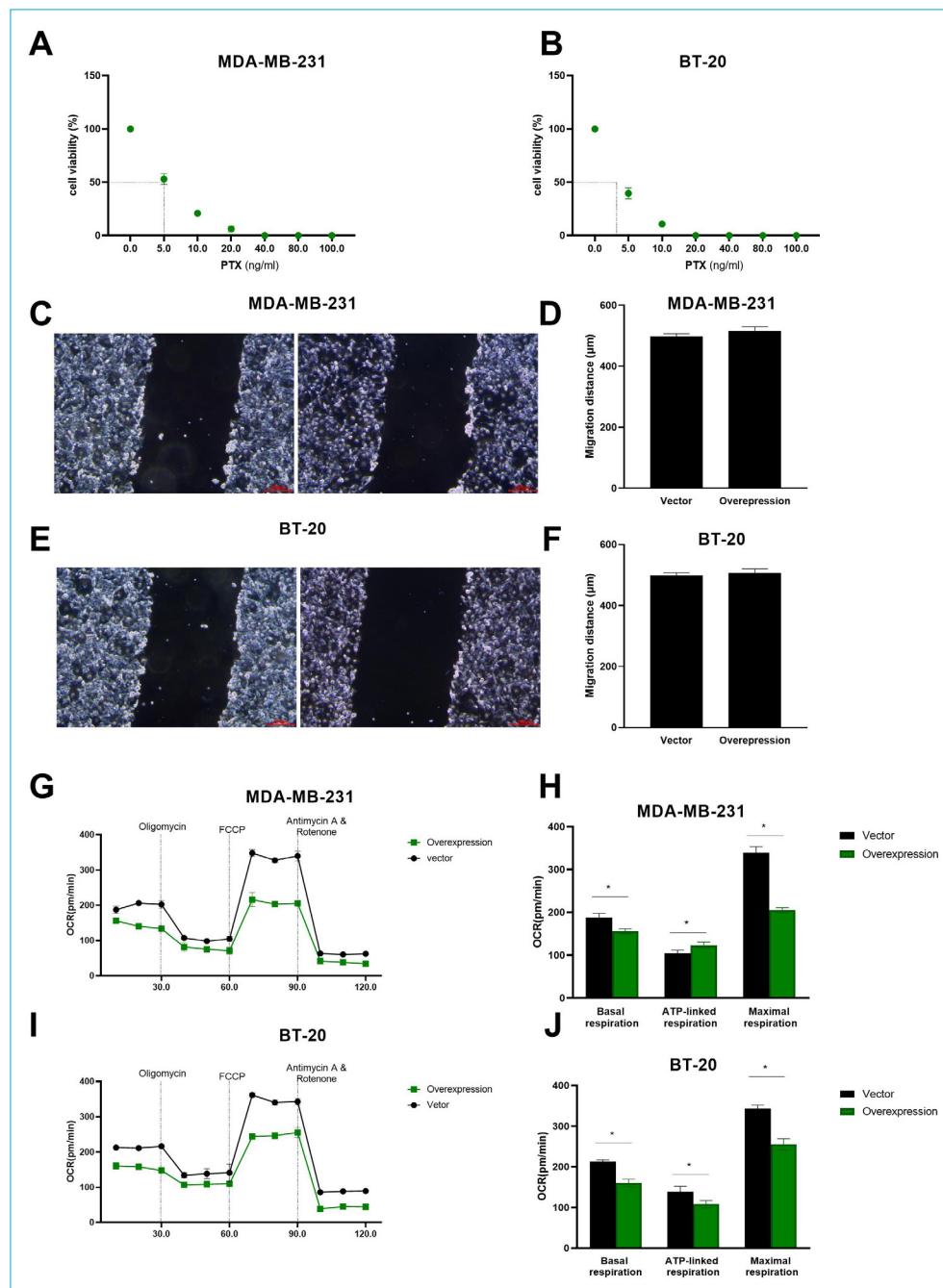




**Figure 3.** Cell scratch assay after NEIL3 silence in MDA-MB-231 and BT-20 cells. A-B Image of cell scratch assay. C-D. Data statistics for cell scratch assays. The NC group was the transfer the sequence which was no influence on the cells.



**Figure 4.** The silencing of NEIL3 inhibits mitochondrial respiratory functions in MDA-MB-231 and BT-20 cells via cellular oxygen consumption rates (OCR). A&C. MDA-MB-231 and BT-20 cells were transfected with NC lentivirus or NEIL3 lentivirus with or without PTX treatment to induce mitochondrial dysfunction. B&D. Basal respiration, ATP-linked respiration, and maximal respiration. The NC group (control group) was the transfer the sequence which was no influence on the cells.

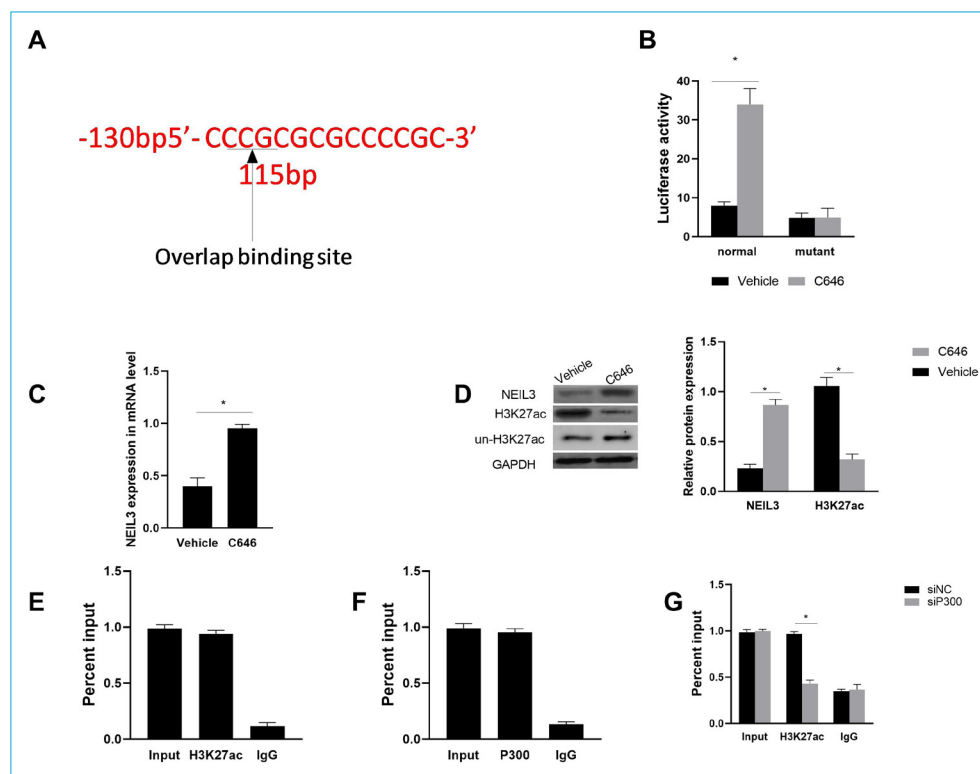


**Figure 5.** NEIL3 overexpression promotes cell proliferation and mitochondrial respiration in MDA-MB-231 and BT-20 cells. MDA-MB-231 and BT-20 cells were transduced with control pLVX-Puro lentivirus or NEIL3 overexpression lentivirus with or without PTX treatment. A-B. Cell ability (IC<sub>50</sub>). C-F. Cell scratch assay. G-J OCR. The vector group was the control. The vector group only has the plasmid, but did not have the NEIL3 sequence.

found that NEIL3 was regulated by P300-mediated modifications of H3K27ac and regulated PTX resistance. NEIL3 was related to clinical treatment chemotherapy sensitivity.<sup>[13,14]</sup> The silencing of P300 inhibited H3H27ac, enhanced interactions between H3K27ac and NEIL3 promoter inhibition, and elevated the expression of NEIL3. This finally in-

hibited NEIL3 expression, increased OCR, accelerated the invasion, and promoted PTX resistance.

OCR has been used as an indicator of mitochondrial function.<sup>[31,32]</sup> We found that basal OCR and ATP-linked OCR were increased following the silencing of *NEIL3*. In addition, basal energy consumption was increased in TNBC



**Figure 6.** P300 modifications of H3K27ac regulates NEIL3. A. NEIL3 promoter mutant. B. Luciferase activity of NEIL3 promoter and NEIL3 promoter mutant. C-D. NEIL3 and H3K27ac expression in MDA-MB-231 resistant PTX cells with or without P300 inhibitor C646 (25  $\mu$ M). E. H3K27ac and NEIL3 promoters in MDA-MB-231-resistant PTX cells. F. P300 and NEIL3 promoters in MDA-MB-231-resistant PTX cells. G. The interaction between H3K27ac and NEIL3 promoters by ChIP assay in MDA-MB-231-resistant PTX cells transfected with control siRNA (siNC) or P300 siRNA (siP300). A: normal (control group) has not any mutant. B&C. vehicle (control group) was not added the C646. D-F. Input (control group) was sonicated, but ChIP was not performed, including the total DNA after sonication of the sample.

cells with PTX resistance with enhanced ATP synthesis. The maximal respiration increased when *NEIL3* was silenced, indicating activated mitochondria in TNBC cells with PTX resistance. Hence, NEIL3 inhibited mitochondrial functions and therefore could be a potential therapeutic target for TNBC resistance.

Mitochondria are critical for cellular energy production and are involved in several cellular processes, including apoptosis and response to chemotherapy drugs such as PTX. Inhibition of mitochondrial respiration can reduce ATP production, thereby affecting the ability of cancer cells to undergo apoptosis, a common mechanism by which chemotherapy drugs induce cell death. PTX is a widely used chemotherapy drug that functions by stabilizing microtubules, preventing cell division, and inducing apoptosis in cancer cells. Resistance to PTX can develop in TNBC cells through several mechanisms, including alterations in drug uptake, increased drug efflux, changes in microtubule dynamics, and activation of survival signaling pathways. The inhibition of mitochondrial functions by NEIL3, an enzyme

involved in DNA repair and redox homeostasis, could potentially contribute to PTX resistance by affecting the energy status of the cell and its ability to undergo apoptosis. For example, reduced mitochondrial function causes a shift toward glycolysis for energy production, a phenomenon known as the Warburg effect and frequently observed in cancer cells and can contribute to chemoresistance. Moreover, the inhibition of mitochondrial respiration could produce reactive oxygen species (ROS), thereby generating oxidative stress. Although ROS are generally associated with cell death, certain cancer cells can adapt to high levels of oxidative stress and utilize it as a survival mechanism, potentially contributing to drug resistance.

Acetylation is one of the common epigenetic histone modifications. Histone acetyltransferases exert different functions in tumorigenesis, depending on their acetylation sites.<sup>[33,34]</sup> The elevated expression of H3K27ac in esophageal squamous cell carcinoma is higher, and H3K27ac promotes proliferation and migration by activating the CCAT1.<sup>[18, 35,36]</sup> H3K27ac contains several non-coding RNAs in the



promoters, which enhances chemoresistance.<sup>[37]</sup> We have previously reported that the downregulation of p300 effectively inhibits the proliferation of PTX-resistant TNBC cells.<sup>[38]</sup> In this study, we found that NEIL3 is regulated by P300-mediated modifications of H3K27ac. P300 is recruited to the promoter region of NEIL3 through acetylation of H3K27ac, synergistically inhibiting the transcription and expression of NEIL3. These findings imply that the silencing of P300 reduces PTX resistance, invasion, and OCR because of the high expression of NEIL3 in TNBC cells with PTX resistance.

The limitation of this study is that we only demonstrated the function of NEIL3 in cells. An in vitro test could not be included. Especially, the function of NEIL3 in the TNBC PTX resistance need explored further. Meanwhile, the regulated mechanism of NEILs on the PTX resistance need explored further. Thus, the mechanism of NEIL3 warrants further exploration. In summary, in this study, the P300/H3K27ac/NEIL3 axis is highly crucial for PTX resistance in TNBC.

## Conclusion

NEIL3 inhibits PTX resistance in MDA-MB-231 and BT-20 cells. The NEIL3 expression is regulated by P300 modifications of H3K27ac. P300 is recruited to the promoter region of NEIL3 through the acetylation of H3K27ac, synergistically inhibiting the transcription and expression of NEIL3, ultimately inducing PTX resistance in TNBC. The P300/H3K27ac/NEIL3 axis is highly crucial for PTX resistance in TNBC.

## Disclosures

**Ethics Committee Approval:** This study was conducted in accordance with the Declaration of Helsinki and approved by the Research Ethics Committee of Inner Mongolia Medical University (NO. YDK2020021025), and informed consent was obtained from all participants. All methods were carried out in accordance with relevant guidelines and regulations.

**Peer-review:** Externally peer-reviewed.

**Conflict of Interest:** None declared.

**Funding:** National Natural Science Foundation of China (grant no. 82060530).

**Authorship Contributions:** Concept – X.M.W.; Design – X.M.W.; Supervision – Y.F.Z.; Materials – Y.F.Z.; Data collection &/or processing – Y.F.Z.; Analysis and/or interpretation – Y.F.Z.; Literature search – Y.F.Z.; Writing – Y.F.Z.; Critical review – Y.F.Z.

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