HDAC5-mediated deacetylation and nuclear localisation of SOX9 is critical for tamoxifen resistance in breast cancer

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BACKGROUND: Tamoxifen resistance remains a significant clinical challenge for the therapy of ER-positive breast cancer. It has been reported that the upregulation of transcription factor SOX9 in ER+ recurrent cancer is sufficient for tamoxifen resistance. However, the mechanisms underlying the regulation of SOX9 remain largely unknown.

METHODS: The acetylation level of SOX9 was detected by immunoprecipitation and western blotting. The expressions of HDACs and SIRTs were evaluated by qRT-PCR. Cell growth was measured by performing MTT assay. ALDH-positive breast cancer stem cells were evaluated by flow cytometry. Interaction between HDAC5 and SOX9 was determined by immunoprecipitation assay.

RESULTS: Deacetylation is required for SOX9 nuclear translocation in tamoxifen-resistant breast cancer cells. Furthermore, HDAC5 is the key deacetylase responsible for SOX9 deacetylation and subsequent nuclear translocation. In addition, the transcription factor C-MYC directly promotes the expression of HDAC5 in tamoxifen resistant breast cancer cells. For clinical relevance, high SOX9 and HDAC5 expression are associated with lower survival rates in breast cancer patients treated with tamoxifen.

CONCLUSIONS: This study reveals that HDAC5 regulated by C-MYC is essential for SOX9 deacetylation and nuclear localisation, which is critical for tamoxifen resistance. These results indicate a potential therapy strategy for ER+ breast cancer by targeting C-MYC/HDAC5/SOX9 axis.

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BACKGROUND

Although endocrine therapy by using oestrogen antagonist tamoxifen can effectively improve the survival rates of oestrogen receptor-alpha (ERα) positive breast cancer patients, however a large proportion of patients are resistant to tamoxifen therapy. Tamoxifen resistance remains a significant clinical obstacle, and there is an urgent need to clarify the relapse mechanisms after the endocrine therapy.

Recently, cancer stem cell-related transcription factor SOX9 located in the nucleus has been shown to cause endocrine-therapy resistance. SOX9 belongs to SRY-related high-mobility-group box (SOX) protein family, which is comprised by a group of transcriptional regulators that have a highly conserved high-mobility group (HMG) domain similar to that of sex-determining region Y protein (SRY) that mediates DNA binding function. Several SOX proteins have been identified as critical transcription factors (TFs) involved in embryonic development and cell fate decision, such as SOX9, which was reported to be essential for cartilage differentiation. Cartilage cells with cytoplasmic SOX9 exhibit impaired transcriptional activation of two well-characterised SOX9 target genes, including collagen type IIα1 and β-catenin for chondrocyte differentiation. In addition, SOX9 is also involved in tumour invasion and metastasis, attenuating therapeutic sensitivity and promoting stem cell proliferation. SOX9 enhances the liver cancer stem cells (CSCs) self-renew through maintaining non-symmetry cell dividing. In human breast cancer, previous study has shown that cytoplasmic accumulation of SOX9 is significantly correlated with enhanced proliferation in invasive ductal carcinoma and metastatic breast cancer. However, in ER+ breast cancer cell, SOX9 is localised in the nucleus. Notably, SOX9 nuclear expression is upregulated in tamoxifen resistant breast cancer (TAMR) cell that is sufficient to cause endocrine resistance. Studies have shown that SIRT1-mediated deacetylation of SOX9 renders its nuclear localisation, which sequentially facilitates SOX9 binding to the promoters of its target genes. However, so far, how SOX9 subcellular localisation is regulated remains largely unknown, especially in TAMR cells.

Protein acetylation and deacetylation have been defined as important post-translational modification processes for the activity, stability and subcellular localisation of proteins. HDACs constitute a family of protein deacetylases that remove acetyl...
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METHODS

Reagents and antibodies
Rabbit polyclonal anti-SOX9 (Millipore, AB5535), Mouse anti-Flag tag (Abmart, M20008), Mouse anti-MYC tag (Abmart, M20012), Mouse monoclonal anti-GAPDH (Beyotime, AF0006), Rabbit polyclonal anti-HDAC5 (CST, 20458), Rabbit anti-C-MYC (D3N8F) (CST, 9402), Rabbit monoclonal Anti-C-MYC (phospho S62) (Abcam, ab185655), Rabbit anti-Di-Methyl-Histone H3 (Lys27) (CST, 9728), Anti-Acetylated Proteins antibody (Abcam, ab193), Rabbit anti-Akt (pan) (C67E7) (CST, 4691), Rabbit anti-phospho-AKT (CST, 13038), Rabbit anti-phospho-p38 MAPK (D13E1) (CST, 9212), Rabbit anti-phospho-p38 MAPK (Thr180/Tyr182) (3D7) (CST, 9215), Rabbit anti-phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) (CST, 4370), Rabbit anti-phospho-p44/42 MAPK (Erk1/2) CST 4695 Rabbit anti-p44/42 MAPK (CST, 9402), Rabbit anti-Flag tag (Abmart, M20008), Rabbit anti-C-MYC tag (Abmart, M20012), Mouse monoclonal Anti-C-MYC (phospho S62) (Abcam, ab185655), Rabbit anti-Di-Methyl-Histone H3 (Lys27) (CST, 9728), Anti-Acetylated Proteins antibody (Abcam, ab193), Rabbit anti-Akt (pan) (C67E7) (CST, 4691), Rabbit anti-Src (36D10) (CST, 2109), Rabbit antiphospho-Src (Tyr416) (CST, 6943), Mouse anti-HER2 (Invitrogen, AHO1011), phospho-HER2 (Tyr1248) (CST, 2247), Rabbit anti-ABC2 (CST, 42078), Rabbit anti-ErbA (CST, 8644), Rabbit anti-Bcl2 (ProteinTech, 12789-1-AP), Rabbit anti-p21 (ProteinTech, 10355-1-AP), Rabbit anti-ALDH1A1 (CST, 36671T), Rabbit anti-Sox2 (CST, 35795), Mouse anti-CD44 (CST, 3570), Rabbit anti-Flag-M2 Magnetic Beads (Sigma, M8283), Goat anti-Rabbit IgG (H+L) Secondary Antibody, Alexa Fluor 594 (Invitrogen, R37117), Goat anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 488 (Invitrogen, A-11034), Thiazolyl Blue Tetrazolium Bromide (Sigma M2128), ALDEFLUOR™ Kit (STEM CELL, #01700), Dual luciferase assay (Promega, 0000170721) and SimpleChiP Assay Kits (CST, 9005): MS257, EX527 and LMK-235 (Selleck).

Cell lines and cell culture
MCF-7, T47D and MDA-MB-231 cells were cultured by standard procedures of American Type Culture Collection. MCF-7, MDA-MB-231 and T47D cells were maintained in DMEM medium, supplemented with 10% foetal bovine serum under humidified atmosphere containing 5% CO2 at 37 °C. For TAMR T47D cells, T47D cells were cultured with tamoxifen for 20 passages with tamoxifen (6 μM). For TAMR T47D cells, MCF-7 cells were cultured with tamoxifen (1 μM) for 12 months.

Quantification of ALDH-positive cells
The ALDEFLUOR kit was used to determine the percentage of cells with high ALDH activity following the manufacturer’s protocol (ALDEFLUOR Stem Cell Technologies, 01700). In brief, cells were washed with phosphate-buffered saline and re-suspended in ALDEFLUOR assay buffer (2 x 10^5 cells/ml) and incubated with ALDEFLUOR substrate with or without ALDH inhibitor, diethylaminobenzaldehyde (DEAB) for 45 min at 37 °C in a water bath. Fluorescence-activated cells were analysed and determined the percentage of ALDH-positive cells in the samples.

Immunofluorescence
The cells were washed three times with PBS and fixed for 15 min at room temperature with 4% (vol/vol) paraformaldehyde. The primary antibody, anti-SOX9 (1:250 dilution, Millipore), was used. Fixed cells were rinsed with PBS and then were incubated for 10 min on ice with 0.2% Triton X-100 and 0.2% BSA in PBS. Following punching, non-specific binding in the cells was blocked by incubation for 30 min at room temperature with 0.3% Triton X-100 and 5% BSA in PBS, and cells were incubated overnight at 4 °C with specific primary antibodies (identified above). After three washes with PBS, the cells were incubated for another 2 h with secondary antibodies. Subsequently, the cells were washed three times with PBS and were stained with PBS containing DAPI (Sigma). All images were collected with a confocal microscope (Nikon A1R, Japan).

Immunoblot and immunoprecipitation assays
For immunoprecipitation, whole-cell extracts were lysed in IP Lysis Buffer (Pierce, 77875) and a protease inhibitor ‘cocktail’ (Sigma, P8340). Cell lysates were centrifuged for 15 min at 12,000 x g. Supernatants were collected and incubated with protein G magnetic beads (Invitrogen, 04555858) together with specific antibodies. After overnight incubation, protein G magnetic beads were washed five times with IP wash buffer. Immunoprecipitation was eluted by SDS-PAGE loading buffer or Elution buffer (Pierce, 88848). For immunoblot analysis, cells were lysed with cell lysis buffer (CST, 9803) supplemented with a protease inhibitor ‘cocktail’. Protein concentrations in the extracts were measured by BCA assay (Pierce, 23235). Equal amounts of extracts were separated by SDS-PAGE, then transferred onto polyvinylidene fluoride membrane (Millipore, IPVH00010), blocked with 5% dry nonfat milk in Tris-buffered saline (pH 7.4) containing 0.1% Tween-20 and probed with the antibody for immunoblot analysis.

Chromatin immunoprecipitation
Chromatin immunoprecipitation (ChIP) was performed by using a ChIP assay kit according to the manufacturer’s protocol (CST, 9003). Cells (2 x 10^7) were cross-linked by formaldehyde. The ChIP-enriched DNA samples were quantified by real-time PCR, and the data are presented as the percentage of input. The primers used were as follows: forward 5’-ATTTTTAGCTCAGGGTCGGG-3’; reverse 5’-GCAGCCTGCTCCTCACATCTTTG-3’. Dual-luciferase reporter assay
Dual-luciferase reporter assay was performed in 96-well plates by using a Dual-Luciferase/Renilla Reporter Assay System (Promega, Madison, WI) as described by the manufacturer. pRL-TK (Renilla luciferase) was used as an internal control. A total of 0.2 μg of DNA was used throughout.

METABRIC dataset analysis
We evaluated overall survival (OS) defined as the intervals between the date of diagnosis for two genes HDAC5 and SOX9. We extracted normalised gene expression data for both genes from a public clinical database METABRIC (Molecular Taxonomy of Breast Cancer International Consortium). Expression data were analysed as continuous variables. We used Cox regression to quantify hazard ratio (HR) and the corresponding confidence interval (CI) in the models that included adjustment for age at diagnosis and TNM stage. This analysis was conducted by using SAS (version 9.4; SAS Institute, Inc., Cary, NC).
HDAC5 is essential for the nuclear localisation of SOX9 in TAMR cells

It has been previously reported that SOX9 located in the cytoplasm abrogates the growth arrest response of HDAC inhibitors in basal-type breast cancer cells. To determine the effect of HDAC inhibitors on SOX9 localisation in TAMR cells, we used pan-HDAC inhibitor Trichostatin A (TSA) to treat TAMR cells. TSA treatment induced cytoplasm sequestration of SOX9 (Fig. 2a and Supplementary Fig. 1F) and inhibited TAMR cell growth (Fig. 2b and Supplementary Fig. 1G) both in MCF-7 and T47D TAMR cells. Moreover, the molecular weight of SOX9 in the cytoplasm was higher than that in the nucleus, suggesting that the post-transcriptional modification of SOX9 by HDAC5 is crucial for SOX9 nuclear localisation.

It has been identified that acetylation reduces SOX9 nuclear entry in chordocytes. Sirtuin 1 (SIRT1) is crucial for SOX9 deacetylation in cartilage cells. We, therefore, detected whether SIRT1 was also involved in the deacetylation of SOX9 in TAMR MCF-7 cells. As shown, SOX9 could not interact with SIRT1 in TAMR MCF-7 cells (Fig. 2c). SIRT1 inhibitor EX527 treatment also did not change the SOX9 localisation in TAMR cells (Fig. 2d), indicating that SIRT1 was not involved in SOX9 deacetylation and nuclear translocation in TAMR MCF-7 cells.

We then treated TAMR MCF-7 cells with different HDAC inhibitors to identify which deacetylase was involved in SOX9 deacetylation. Treatment with MS275, an inhibitor of HDAC1 and HDAC3, failed to affect SOX9 nuclear localisation in TAMR cells (Fig. 2e). Interestingly, LMK-235, which is an HDAC4 and HDAC5 inhibitor, enhanced SOX9 cytoplasm localisation (Fig. 2f and Supplementary Fig. 1H). To further explore the key member of HDACs that mediates the deacetylation of SOX9, we used immunoprecipitation assay to identify the molecules that associate with SOX9. Given that LMK-235 inhibits the activations of HDAC4 and HDAC5, we then focused on HDAC4 and HDAC5. Immunoprecipitation analysis in MCF-7 TAMR cells showed that SOX9 interacted with HDAC5 but not HDAC4 (Fig. 2h). Interestingly, we compared the expression of 18 human deacetylase enzymes in MCF-7 parental and TAMR cells, in which only HDAC5 was significantly upregulated in TAMR cells compared with parental cells (Fig. 2i). Taken together, these results indicate that HDAC5 plays an important role in mediating SOX9 nuclear localisation in TAMR cells.

HDAC5 is indispensable for deacetylation of SOX9 in TAMR cells

We have found that HDAC5 inhibitor could induce SOX9 cytoplasm localisation, prompting us to test whether HDAC5 is a major contributor for the acetylation and function of SOX9 in TAMR cells. Knocking down HDAC5 promoted cytoplasm localisation of SOX9 in MCF-7 and T47D TAMR cells (Fig. 3a, b and Supplementary Fig. 2A) which was also consistent with immunofluorescence staining results shown in Fig. 3c and Supplementary Fig. 2B. Significantly, knocking down HDAC5 increased the level of SOX9 acetylation by detecting the acetylation status of SOX9 with anti-acetylation antibody (Fig. 3d and Supplementary Fig. 2C). These results suggest that HDAC5 is dispensable for SOX9 deacetylation in TAMR cells.

We then further explored the function of HDAC5 in tamoxifen resistance. The growth of TAMR cells was significantly reduced after knocking down HDAC5 expression (Fig. 3e and Supplementary Fig. 2D). Similarly, LMK-235 treatment also reduced the growth of both MCF-7 and T47D TAMR cells (Fig. 3f and Supplementary Fig. 2E). Besides, knocking down HDAC5 also decreased the percentages of ALDH-positive cell population in TAMR cells (Fig. 3g). Consistent with the results from the SOX9-knockout cells, CSC-related molecules CD44, ALDH1A1 and SOX2 were reduced when knocking down HDAC5 in TAMR MCF-7 cells (Fig. 3h). Conversely, overexpression of HDAC5 in MCF-7 promoted the cell growth while treated with tamoxifen (Fig. 3i).
results were observed in T47D TAMR cells (Supplementary Fig. 2F).

To examine the clinical relevance of HDAC5 in breast cancer, its expression values were analysed by using publicly available microarray data resources. Public dataset analysis (GEO: GSE9574) revealed that the expressions of HDAC5 in tumour tissues of ER+ breast cancer patients were higher than those in the epithelium adjacent to breast tumours (Fig. 3j). We further evaluated the associations between overall survival (OS) and two genes HDAC5...
and SOX9 expression levels in endocrine-therapy-treated ER-positive cases from the METABRIC. The sample size is 1178 after excluding those participants who do not have TNM data. There are 503 deaths and the median survival is 7.53 years. We used combined genes of HDAC5 and SOX9 expression levels as an exposure in the calculation and found that the $P$-value was 0.0409 (95% CI: 0.82–0.99, $P = 0.0409$) (Fig. 3k). Thus, HDAC5 and SOX9 expression levels are crucial factors related to the overall survival in endocrine-therapy-treated ER$^+$ breast cancer.

Collectively, these results suggest that HDAC5 deacetylates SOX9 to maintain its nuclear localisation, which drives tamoxifen resistance in breast cancer. HDAC5 and SOX9 are related to the poor survival rates in endocrine-therapy-treated ER$^+$ breast cancer.

Given that HDAC5 is critical for mediating the deacetylation of SOX9, we then tried to confirm that SOX9 directly interacts with HDAC5. The interaction between SOX9 and HDAC5 was observed.
We next examined the interaction between endogenous SOX9 and HDAC5. SOX9 was shown to be associated with HDAC5 in MCF-7 and T47D TAMR cells (Fig. 4c, d). Thus, these results clearly indicated that there is an interaction between SOX9 and HDAC5 in TAMR cells. Moreover, stronger interaction between SOX9 and HDAC5 was found in TAMR cells than that in parental cells of MCF-7 and T47D (Fig. 4e, f). To identify which domain of SOX9 was

in MCF-7 cells ectopically expressing SOX9 and HDAC5 (Fig. 4a, b). We next examined the interaction between endogenous SOX9 and HDAC5. SOX9 was shown to be associated with HDAC5 in MCF-7 and T47D TAMR cells (Fig. 4c, d). Thus, these results clearly indicated that there is an interaction between SOX9 and HDAC5 in TAMR cells. Moreover, stronger interaction between SOX9 and HDAC5 was found in TAMR cells than that in parental cells of MCF-7 and T47D (Fig. 4e, f). To identify which domain of SOX9 was
required for its interaction with HDAC5, two truncated mutants of SOX9 were constructed: HMGB domain (1–181 aa) mutant and C-terminal domain (TA domain) (182–507 aa) mutant. The results illustrated that HMGB domain of SOX9 is responsible for its interaction with HDAC5 (Fig. 4g). Then, the truncated mutants of HDAC5 were constructed: MEF2 binding domain (1–200 aa) mutant, NLS domain (200–498 aa) mutant and HDAC domain (498–1123 aa) mutant. The results indicated that HDAC domain of HDAC5 is critical for its interaction with SOX9 (Fig. 4h). Collectively, these data demonstrate that HDAC domain of HDAC5 binds to HMGB domain of SOX9 in TAMR cells.

C-MYC directly promotes HDAC5 transcription

Although HDAC5 is highly expressed in TAMR cells and promotes tamoxifen resistance, the regulatory mechanism for HDAC5 upregulation in tamoxifen-resistant cells is not well understood.
Online prediction tool PROMO was used to analyze the potential transcription factor-binding sites in HDAC5 promoter sequences. Among the 65 potential transcription factors, we speculated that C-MYC may be the key transcription factor for promoting HDAC5 transcription, as the expression of C-MYC is positively correlated with HDAC5 expression indicated by analysis with the public dataset GSE9574 in ER+ breast cancer (Fig. 5a). As it has been reported that C-MYC is crucial for tamoxifen resistance in breast cancer to overcome the growth reduction,28 we then measured C-MYC activation in TAMR MCF-7 cells. We confirmed that the protein levels of both C-MYC and phosphorylated C-MYC (Serine 62) were higher in MCF-7 TAMR cells compared with parental cells (Fig. 5b). When knocking down C-MYC in TAMR cells, HDAC5 expression was significantly reduced, while other class I and II HDACs and class III SIRTs were not affected (Fig. 5c–f). Conversely, ectopically expressing C-MYC in MCF-7 TAMR cells increased HDAC5 expression at both mRNA and protein levels (Fig. 5g, h).

Given that C-MYC expression is positively related with HDAC5 expression, we then aimed to explore whether C-MYC is a transcription factor for HDAC5 and directly regulates its expression. Sequence analysis showed that the promoter of HDAC5 contains the typical sequence of C-MYC binding sequence (CACGTG), which is located approximately −100 bp away from the upstream of the transcription start point. To further confirm the association between C-MYC and HDAC5, we used chromatin immunoprecipitation (CHIP) to detect whether C-MYC bound to the promoter of HDAC5. We designed primers for the region of −200/+20 bp upstream of the transcription start point of HDAC5. The results showed that C-MYC bound to the region of −200/+20 bp upstream of the transcription start point of HDAC5 in TAMR cells (Fig. 5i). The plasmid containing −200/+20 bp upstream of the transcription start point of HDAC5 was constructed, and consistent results were obtained from dual-reporter luciferase assay, which confirmed that C-MYC activates the expression of HDAC5 (Fig. 5j).

Collectively, these findings demonstrate that C-MYC is a transcription factor for HDAC5 and directly promotes its transcription in TAMR cells.
C-MYC maintains SOX9 nuclear localisation in TAMR cells. Based on the fact that HDAC5 is indispensable for SOX9 nuclear localisation in TAMR cells, we speculated that C-MYC is required for the localisation and function of SOX9. Knocking down C-MYC inhibited SOX9 nuclear localisation that was shown by immunofluorescence staining in MCF-7 and T47D TAMR cells (Fig. 6a and Supplementary Fig. 3A). Nuclear and cytoplasm proteins were separated to confirm that SOX9 mostly localised in the cytoplasm in shC-MYC TAMR MCF-7 cells (Fig. 6b and Supplementary Fig. 3B). Then we used immunoprecipitation assay to detect the acetylation status of SOX9. Using anti-acetylation antibody for western blot revealed that knocking down C-MYC induced a higher acetylation level of SOX9 (Fig. 6c and Supplementary Fig. 3C). We next determined the effect of C-MYC on the expression of two SOX9 target genes, TCF4 and HMGA2. The results showed that there was significant reduction in the expression levels of these target genes when knocking down C-MYC in TAMR cells (Fig. 6d and Supplementary Fig. 3D). In addition, knocking down C-MYC reduced the growth of TAMR cells (Fig. 6e and Supplementary Fig. 3E). MTT assay showed that HDAC5 rescued the reduced growth rate in shC-MYC MCF-7 TAMR cells (Fig. 6f). Ectopically expressing HDAC5 in shC-MYC MCF-7 cells translocated SOX9 back into the nucleus (Fig. 6g and Supplementary Fig. 3F). As reported, the deacetylation of 249 lysines of SOX9 is crucial for its nuclear localisation, we constructed full-length SOX9 with 249 lysine mutations replaced by glycine (KA-249). Transfecting KA-249 SOX9 into shC-MYC TAMR cells could restrict SOX9 to localising in the cytoplasm, while wild-type SOX9 was still localised in the cytoplasm (Fig. 6h). Transfeting KA-249 SOX9 rescued the SOX9-KO-induced growth suppression in MCF-7 TAMR cells but not the wild type of SOX9 (Fig. 6i).

Thus, these results indicate that C-MYC/HDAC5/SOX9 axis plays a critical role in maintaining tamoxifen resistance in breast cancer, which might be the potential target for reversing the tamoxifen resistance.
DISCUSSION

In this study, we provided the evidence that HDAC5 is indispensable for SOX9 deacetylation and nuclear translocation, and C-MYC is the transcription factor that directly promotes the expression of HDAC5. Therefore, C-MYC/HDAC5/SOX9 axis is essential for promoting tamoxifen resistance in breast cancer.

Protein acetylation, which was firstly found in histone lysine residues, mainly regulates gene transcription. Acetylation of non-histone proteins, such as p53, STAT3 and EZH2, also has been reported to play important roles in diverse physiological processes. Acetylation is a dynamic process and can be reversed by specific deacetylases. The opposite effects of histone acetyltransferases (HATs) and HDACs allow gene expression to be exquisitely regulated through chromatin remodelling and post-translational modifications. The modifications and localisation of SOX9 protein are important for its various functions in different cell types. The characteristics of distributions indicate that SOX9 has different functions between ER$^+$ and basal-type breast cancers. SOX9 localised in the cytoplasm loses the function as a transcription factor for the expression of target genes. Here we report that SOX9 localisation is also affected by HDAC inhibitors in TAMR cells.

We treated TAMR cells with several selected inhibitors for HDACs and SIRTs. HDAC4 and HDAC5 inhibitor LMK-235 is able to render SOX9 to localise into the cytoplasm to decrease the proliferation of TAMR cells. We then identified that HDAC5 is critical for SOX9 nuclear localisation. In addition to SOX9, HDAC5 has also been previously reported to be able to deacetylate other non-histone proteins. In basal-type breast cancer, depletion of HDAC5 by shRNA not only hinders cell proliferation, manifesting as arresting G1 cell cycle, but also attenuates migration and colony formation of breast cancer cells through regulating LSD1 protein stability by decreasing LSD1 protein acetylation. Studies have also shown that deacetylase activity of HDAC5 is required for HIF-1α nuclear accumulation. In the present study, we found that HDAC5 is indispensable for SOX9 deacetylation. For clinical relevance, breast cancer patients with tamoxifen treatment expressing elevated SOX9 and HDAC5 have a worse overall survival. These results suggest that HDAC5 and SOX9 are involved in regulation of tumour progression after tamoxifen treatment. However, the correlation between SOX9 acetylation level and overall survival of tamoxifen-resistance breast cancer patients still needs more clinical evidence.

Although suitable endocrine therapy with tamoxifen or aromatase inhibitor can effectively increase the survival rate of breast cancers, studies have clarified many molecular mechanisms that cause endocrine-therapy resistance. There is an urgent requirement for an in-depth study of the relapse mechanisms for the endocrine therapy. Despite the promising clinical results produced by HDAC inhibitors in treatment of haematological malignancies such as T-cell lymphoma, no apparent clinical evidence indicates that HDAC inhibitors work effectively as a monotherapy against solid tumours including breast cancer. Here, we demonstrated that HDAC5 promotes the growth of TAMR cells and decreases the overall survival of breast cancer patients with tamoxifen treatment, which makes HDAC5 a potential therapeutic target for the reverse of tamoxifen resistance. The potential therapeutic value of combining tamoxifen with HDAC inhibitors is worth for clinical observation.

The complex interplays among transcription factors are essential for development and progression of breast cancer to a lethal disease. For example, the transcription factor FOXK2 represses the proliferation and invasion of breast cancer cells via reciprocal successive feedback by transcription factor HIF-1β. It has been shown that C-MYC is a crucial transcription factor for breast cancer progression and tamoxifen resistance. In this report we uncovered the connection between the transcription factors SOX9 and C-MYC. C-MYC increases the expression of HDAC5 that is dispensable for SOX9 deacetylation and tamoxifen resistance.

In summary, in the present study we identified HDAC5, whose transcription promoted by C-MYC, is essential for SOX9 deacetylation and nuclear localisation in tamoxifen-resistant breast cancer. Our novel findings also provide supportive evidence that an orchestrated interaction between HDAC5 and SOX9 is a critical mechanism to enhance transcriptional activities of tamoxifen-resistant related genes. Targeting C-MYC/HDAC5/SOX9 axis might be beneficial for the discovery of new strategies for ER$^+$ breast cancer therapy.

AUTHOR CONTRIBUTIONS

O.W, Y.X. and L.L. designed the research. Y.X., J.Z., W.L., W.Y., Q.L., Z.L. and H.Q. performed experiments, acquisition and analysis of data. Y.X., L.L. and Q.W. wrote the paper. J.G., X.G. and W.L. analysed the dataset of METABRIC. All authors have read and approved the paper.

ADDITIONAL INFORMATION

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Competing interests: The authors declare no competing interests.

Ethics approval and consent to participate: The human data involved in current study are obtained and analysed from the publicly archived datasets. There are no animal data in current study.

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Consent to publish: Consent is obtained from all authors. All subjects gave written informed consent for publication.

Data availability: The datasets used and analysed during current study are available from the corresponding authors on reasonable request. The hyperlinks of publicly archived datasets involved in this study are as follows: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE59195; https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE59574; http://molonc.bccrc.ca/aparicio-lab/research/metabric/

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