Nuclear lactate dehydrogenase A senses ROS to produce α-hydroxybutyrate for HPV-induced cervical tumor growth

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It is well known that high-risk human papilloma virus (HR-HPV) infection is strongly associated with cervical cancer and E7 was identified as one of the key initiators in HPV-mediated carcinogenesis. Here we show that lactate dehydrogenase A (LDHA) preferably localizes in the nucleus in HPV16-positive cervical tumors due to E7-induced intracellular reactive oxygen species (ROS) accumulation. Surprisingly, nuclear LDHA gains a non-canonical enzyme activity to produce α-hydroxybutyrate and triggers DOT1L (disruptor of telomeric silencing 1-like)-mediated histone H3K79 hypermethylation, resulting in the activation of antioxidant responses and Wnt signaling pathway. Furthermore, HPV16 E7 knocking-out reduces LDHA nuclear translocation and H3K79 tri-methylation in K14-HPV16 transgenic mouse model. HPV16 E7 level is significantly positively correlated with nuclear LDHA and H3K79 tri-methylation in cervical cancer. Collectively, our findings uncover a non-canonical enzyme activity of nuclear LDHA to epigenetically control cellular redox balance and cell proliferation facilitating HPV-induced cervical cancer development.
HPV type and accounts for ~55% cervical cancer. Among all HR-HPVs, HPV16 is the most carcinogenic and affect the protein functions through nuclear translocation of LDHA and the product of α-redox proteomics method using iodoacetyl-α-HB). The accumulation of LDHA nuclear translocation, we packaged retrovirus containing HPV16 genome, and HeLa (containing HPV18 genome) cells (Supplementary Fig. 1b). The key glycolysis enzyme LDHA was identified to be a potential key regulator in HPV-induced cervical cancer development (Supplementary Data 1).

To investigate the potential role of LDHA in HPV-induced cervical cancer, we collected HPV-negative and HPV-positive cervical cancers from 66 patients (Supplementary Data 2). Immunohistochemistry (IHC) showed that LDHA nuclear staining was significantly increased in HPV16-positive tumors compared with HPV16-negative tumors (Fig. 1a, b). To test whether the pivotal oncprotein HPV16/18 E7-mediated LDHA nuclear translocation, we packaged retrovirus containing HPV16/18 E7 gene and infected primary human cervix keratinocytes (PHKs), immortalized human keratinocyte cell line HaCaT, and transfected HPV16 E7 gene into HPV-negative human cervical cancer cell line HT-3 (Supplementary Fig. 2a). As expected, HPV16/18 E7 expression dramatically increased the percentage of LDHA nuclear-translocated cells from ~5% to ~50% (Fig. 1c, d, and Supplementary Fig. 2b, c). In line with the potential effect of HPV infection on ROS production, we found that HPV16/18 E7 induction resulted in cellular ROS accumulation (Fig. 1e and Supplementary Fig. 2d). Notably, supplement with a ROS scavenger N-acetyl-L-cysteine (NAC) remarkably reduced LDHA nuclear translocation in HPV16/18 E7-transduced cells (Fig. 1c, d, and Supplementary Fig. 2b, c). This observation triggered us to speculate that ROS possibly promote LDHA nuclear translocation. To this end, we treated HaCaT, HT-3, U2OS, and HeLa cells with hydrogen peroxide (H2O2) and found that LDHA rapidly translocated from the cytoplasm to nucleus in a dose-dependent manner, and the H2O2-induced subcellular redistribution of LDHA was reversed by NAC supplement (Fig. 1f, g, and Supplementary Fig. 3a–d). Meanwhile, the cellular ROS levels were measured upon H2O2 and NAC treatment in HT-3 and U2OS cells under the same condition (Supplementary Fig. 3e). To further validate this, we performed nuclear isolation assay and found the similar pattern for LDHA localization (Fig. 1h). These data indicated that LDHA nuclear translocation induced by HPV infection is dependent on ROS.

Mitochondrial respiratory chain and nicotinamide adenine dinucleotide phosphate (NADPH) oxidases (NOXs) contribute the major source of endogenous ROS in mammal. To figure out the potential source of ROS induced by HPV16/18 E7, mitochondrial and NOXs originated ROS were measured, respectively. As shown in Supplementary Fig. 4a, HPV16/18 E7 expression increased mitochondrial ROS generation, as determined using the fluorescent dye MitoSOX Red. On the other hand, NOXs inhibitor diphenyleneiodonium chloride (DPIC) decreased the intracellular ROS level (Supplementary Fig. 4b), suggesting that NOXs contributes to HPV16/18 E7-induced intracellular ROS elevation as well as mitochondrial respiratory chain.

Nuclear LDHA gains a noncanonical activity to produce α-HB. Lactate dehydrogenase acts an indispensable role in cancer development and possesses a well-defined canonical enzyme activity, catalyzing the conversion between pyruvate (Pyr) and lactate (Lac)32. It is well known that LDHA localizes at cytoplasm exerting its lactate-producing activity. Interestingly, LDHA has tandem mass tag (iodoTMT) reagents composed of a sulphydryl-reactive iodoacetyl group selective labeling sulphydryl (-SH) groups and sets of isobaric isomers which can be differentiated by mass spectrometry (MS), enabling quantitation of the relative abundance of cysteine modifications (Supplementary Fig. 1a). To identify HPV-related redox-sensitive effectors, the cysteine proteomes were obtained from C33A (HPV negative), SiHa (containing HPV16 genome), and HeLa (containing HPV18 genome) cells (Supplementary Fig. 1b). The key glycolysis enzyme LDHA was identified to be a potential key regulator in HPV-induced cervical cancer development (Supplementary Data 1).

Results
HPV-induced LDHA nuclear translocation is dependent on ROS. HPV infection is the most common factor of cervical cancer. Among all HR-HPVs, HPV16 is the most carcinogenic HPV type and accounts for ~55%–60% of all cervical cancers, and HPV18 is the second type which accounts for 10%–15%. Persistent oxidative stress is always accompanied by the process of HPV infection to host cells. The altered cellular redox levels may cause reversible modifications on specific cysteine residues and affect the protein functions. To decipher the changes in the cysteine proteome on HR-HPV infection, we developed a sensitive and specific redox proteomics method using iodoacetyl mass spectrometry (MS), enabling quantitation of the relative abundance of cysteine modifications (Supplementary Fig. 1a). To identify HPV-related redox-sensitive effectors, the cysteine proteomes were obtained from C33A (HPV negative), SiHa (containing HPV16 genome), and HeLa (containing HPV18 genome) cells (Supplementary Fig. 1b). The key glycolysis enzyme LDHA was identified to be a potential key regulator in HPV-induced cervical cancer development (Supplementary Data 1).

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also been shown to exhibit noncanonical enzyme activities\textsuperscript{33–35}. We hypothesized that, after nuclear translocation, LDHA probably gained a noncanonical enzyme activity. To this end, we discovered that LDHA acquired an α-HB-producing activity in the nucleus (Fig. 2a and Supplementary Fig. 5). We measured both the canonical and noncanonical enzyme activities upon H\textsubscript{2}O\textsubscript{2} exposure or HPV16 E7 induction, with or without NAC supplement. Interestingly, the noncanonical enzyme activity but not
the canonical activity of LDHA was elevated by ~1.5-fold after HPV16 E7 induction or H2O2 treatment (Fig. 2b, c). Moreover, NAC supplement decreased noncanonical enzyme activity of LDHA, suggesting a ROS-dependent switch in LDHA activity. In addition, we quantified four direct substrates of LDHA, including pyruvate, lactate, α-ketobutyrate (α-KB), and α-HB, using liquid chromatography coupled to triple quadrupole tandem mass spectrometry (LC-MS/MS). As expected, α-HB, the product of noncanonical LDHA activity, accumulated by ~1.6-fold after HPV16/18 E7 induction or H2O2 treatment, while no significant changes were observed on pyruvate or lactate levels (Fig. 2d, e, and Supplementary Fig. 6a–e). Notably, NAC supplement totally blocked the elevation of α-HB (Fig. 2d, e, and Supplementary Fig. 6d, e). To assess the function of nuclear LDHA, we isolated...
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Fig. 2 Nuclear LDHA gains a noncanonical enzyme activity to produce α-HB. a Schematic of the canonical and noncanonical LDHA enzyme activity. b HPV16 E7 enhances the noncanonical enzyme activity of LDHA. The canonical and noncanonical LDHA enzyme activities were measured in HaCaT and HT-3 cells stably expressing vector or HPV16 E7 coupled with or without 1 mM NAC treatment for 6 h. c ROS elevate the noncanonical enzyme activity of LDHA. HaCaT and HT-3 cells were treated with or without 10 μM H2O2 for 6 h, and supplemented with or without 1 mM NAC for extended 6 h as indicated for measurement of the canonical and noncanonical LDHA enzyme activities. d HPV16 E7 expression accumulates cellular α-HB. The extracted metabolite samples from the HT-3 cells stably expressing vector or HPV16 E7 coupled with or without 1 mM NAC treatment for 6 h as indicated were analyzed by liquid chromatography coupled to triple quadrupole tandem mass spectrometry (LC-MS/MS), relative abundance (by metabolite peak area) was shown. e Nuclear LDHA presents higher noncanonical enzyme activity. The canonical and noncanonical LDHA enzyme activities were measured in HeLa stable cells with LDHA knockdown and Vec/WT/NLS/NES rescue. Vec, vector, WT, wild-type; NLS, nuclear localization signal; NES, nuclear export signal. g LDHA nuclear translocation accumulates cellular α-HB. The extracted metabolite samples from HeLa stable cells with LDHA knockdown and Vec/WT/NLS/NES rescue were analyzed by LC-MS/MS, relative abundance (by metabolite peak area) was shown. LDHA enzyme activities were normalized to LDHA protein level. Relative metabolite abundances were normalized to cell number. Results are representative of three independent experiments. All data are shown as mean ± SEM. The p values were determined by two-tailed t-test. The values of p < 0.05 were considered statistically significant. * , ** , and *** denote p < 0.05, p < 0.01, and p < 0.001, respectively. NS means non significant.
Supplementary Fig. 9d). Given that treatment of α-HB to LDHA NES cells restored H3K79 trimethylation, we hypothesized that α-HB produced by nuclear LDHA might take part in the activation of DOT1L through its binding to LDHA. To test this, we found that treatment of cells with α-HB alone increased the binding between endogenous DOT1L and LDHA (Fig. 4g). Overall, these results suggested that α-HB produced by nuclear LDHA induces H3K79 hypermethylation through promoting the interaction between DOT1L and LDHA, which possibly activates the methyl-transferase activity of DOT1L.

**NRF2 is required for LDHA-induced antioxidant responses.** High ROS levels are generally detrimental to cells, and the increased antioxidant capacity becomes vital to maintaining tumor development and cell survival. In addition, aberrant activation of Wnt/β-catenin signaling pathway, which is frequently observed in human cervical cancer, promotes cell proliferation and tumor progression. Interestingly, the activation of DOT1L has been reported to modulate Wnt target genes expression. To test whether the H3K79 hypermethylation induced by HPV16 E7 expression or H2O2 treatment further

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**Fig. 3** ROS disrupt LDHA tetramer formation and promote noncanonical enzyme activity. (a, b) ROS disrupt LDHA tetramer formation. HaCaT and HT-3 cell extracts with or without 10 μM H2O2 treatment were crosslinked by 0.025% glutaraldehyde and analyzed by western blotting using LDHA antibody. Tetrameric, dimeric, and monomeric LDHA were indicated (a). HT-3 cell extracts were prepared from 1 × 10⁷ cells with or without 10 μM H2O2 treatment and passed over the gel filtration column. Fractions were collected every 0.25 ml per tube and analyzed by western blot for LDHA protein. Molecular mass, 158 and 43 kDa marked below the blots, were determined by Gel Filtration Calibration Kit HMW (GE Healthcare). The loading inputs for gel filtration were shown below (b). (c) Dimer LDHA presents the noncanonical enzyme activity. The canonical and noncanonical LDHA enzyme activity assays were measured on tetramer fractions (Fraction #56, #57) and dimer fractions (Fraction #60, #61) separated from gel filtration. (d, e) More dimers form in the LDHANES group compare with that of LDHA NES group. Cell extracts from HEK293T LDHA KO cells expressing Flag-tagged WT, NLS, and NES LDHA were crosslinked by 0.025% glutaraldehyde and analyzed by western blotting using LDHA antibody. Tetrameric, dimeric, and monomeric LDHA were indicated (d). Cell extracts from HEK293T LDHA KO cells expressing Flag-tagged WT, NLS, and NES LDHA were passed over the gel filtration column. Fractions were collected every 0.25 ml per tube and analyzed by western blot for LDHA protein. Molecular mass was determined by Gel Filtration Calibration Kit HMW (GE Healthcare). Results are representative of three independent experiments. All data are shown as mean ± SEM. The p values were determined by two-tailed t-test. The values of p < 0.05 were considered statistically significant. *, **, and *** denote p < 0.05, p < 0.01, and p < 0.001, respectively. NS means non significant.
regulated cellular antioxidant responses or cell proliferation, seven antioxidant genes and three Wnt target genes were subjected to qPCR analysis. As expected, HPV16 E7 expression and H₂O₂ treatment increased the expression of the majority of these genes, which was blocked by NAC (Fig. 5a and Supplementary Fig. 10a, b). Furthermore, ChIP-qPCR assays were carried out to define the histone methylation status of these HPV16/18 E7-induced target genes. We found that HPV16/18 E7 markedly enhanced H3K79 dimethylation level at the SOD1, CAT, CTNNB1, and MYC gene body, while NAC supplement reversed...
target genes (Fig. 5c and Supplementary Fig. 11a). This data indicates that NRF2, not HIF-1α.

However, EPZ004777 treatment blocked the upregulation of VEGFA mRNA expression of NQO1 and GCLC, HPV16/18 E7-induced antioxidant genes expression. Increased α-HB potentially rescued the antioxidant ability of LDHA-induced oxidative stress in LDHAWT, LDHANLS, and LDHANES cells in response to H2O2. On one hand, LDHANLS cells showed higher expression levels of these genes under normal condition. On the other hand, LDHAWT but not LDHANES cells showed more pronounced target genes expression in response to H2O2 (Fig. 6d and Supplementary Fig. 12b). Consistently, we observed that LDHANLS cells presented a lower level of ROS (Fig. 6e). Collectively, LDHA nuclear translocation is required for the activation of antioxidant genes and Wnt target genes.

As mentioned above, supplement of α-HB to LDHANES cells remarkably recovered H3K79 trimethylation level. We thus speculated that α-HB potentially rescued the antioxidant ability of LDHANES cells. Interestingly, the addition of α-HB remarkably relieved the ROS accumulation (Fig. 6f). In accordance, α-HB supplement significantly upregulated the expression of antioxidant genes and Wnt target genes (Fig. 6g). α-HB supplement effectively rescued cell growth and colony formation in LDHANES cells under continuous oxidative stress in a dose-dependent manner (Fig. 6h, i, and Supplementary Fig. 12c), suggesting that α-HB contributes to, at least in part, LDHA-mediated overcoming of oxidative stress. Collectively, these observations strongly support that LDHA nuclear translocation, in response to HPV induction or ROS stimulation, maintains redox homeostasis and cell survival under stressed conditions. LDHA gain-of-function that produces the antioxidant metabolite, α-HB, is critical for mediating antioxidant response.

Nuclear LDHA implicates in cervical cancer development. To examine the effect of nuclear LDHA in tumor growth, LDHA KO with vector, LDHAWT, LDHANLS, and LDHANES rescue cells were injected subcutaneously into nude mice. Consistent with previous reports, LDHAWT group showed increased tumor growth compared to vector group, while LDHANLS but not LDHANES group displayed significantly increased tumor growth compared with other groups (Fig. 7a–c). This result suggests that nuclear LDHA promotes tumor growth. In agreement, elevated NRF2, SOD1, MYC, and H3K79 trimethylation levels were observed in LDHANLS xenografts (Fig. 7d).

To examine the effect of HPV16-induced LDHA nuclear translocation in vivo, we adopted a K14-HPV16 transgenic mouse model this effect (Fig. 5b and Supplementary Fig. 10c, d). These data suggest that HPV16/18 E7-induced H3K79 hypermethylation upregulates antioxidant genes and Wnt target genes expression in a ROS-dependent manner. Several crucial transcription factors are involved in cellular redox sensing, such as NRF2, hypoxia-inducible factor 1α (HIF-1α), and nuclear factor kappa B (NF-κB)49,48. To further identify the key effector that directly mediated the antioxidant responses corresponding to high H3K79 methylation level, the expression of NRF2, HIF-1α, and NF-κB target genes were analyzed by qPCR. In response to HPV16 E7 expression and H2O2 treatment, the mRNA expression of NRF2 (NQO1, GCLC), HIF-1α (SLC2A1, VEGFA), and NF-κB (IL-10, TNFβ1) target genes expression were remarkably increased (Fig. 5c and Supplementary Fig. 11a). However, EPZ004777 treatment blocked the upregulation of NRF2 target genes (NQO1 and GCLC), but not HIF-1α or NF-κB target genes (Fig. 5c and Supplementary Fig. 11a). This data indicates that NRF2, not HIF-1α or NF-κB, may be the key transcription factor for HPV16 E7 or H2O2-induced H3K79 hypermethylation. Of note, we confirmed the increase of NRF2 protein level as a molecular marker of the NRF2 pathway activation upon HPV16/18 E7 expression (Supplementary Fig. 11b). We further validate the role of NRF2 in H2O2 or HPV16/18 E7-induced antioxidant genes expression. Increased mRNA expression of NQO1 and GCLC was dramatically impaired by deletion of NRF2 or ML385, a NRF2-specific inhibitor49,48 (Fig. 5d, e, and Supplementary Fig. 11c, d). To further test if LDHA is essential for the antioxidant gene activation, LDHA KO and LDHA, NRF2 double-knockout (DKO) HeLa cells were generated for qPCR analysis. As shown in Fig. 5f and Supplementary Fig. 11e, LDHA KO significantly reduced NQO1 and GCLC expression, but not the expression of HIF-1α or NF-κB target genes, upon H2O2 treatment. In addition, activation of antioxidant genes was markedly decreased in DKO cells (Supplementary Fig. 11f, g). Genetic ablation of LDHA remarkably blocked the increase of H3K79 dimethylation level of SOD1, CAT, CTNNB1, and MYC, while deletion of NRF2 blocked the increase of H3K79 dimethylation level of SOD1 and CAT, but not CTNNB1 and MYC gene body (Fig. 5g). Together, these data unveil a LDHA-DOT1L-NRF2 axis in HPV16/18 E7 and H2O2-induced antioxidant responses.

Nuclear LDHA produced α-HB counteracts with oxidative stress. Given the observed activation of antioxidant and Wnt signaling pathways, we presumed that nuclear LDHA enables cell survival and proliferation under oxidative stress. First, we examined the effect of nuclear LDHA on cell proliferation and colony formation in LDHAWT, LDHANLS, and LDHANES cells, respectively. There were no significant changes in cell growth between LDHAWT, LDHANLS, and LDHANES stable cells under normal condition (Fig. 6a). However, LDHAWT and LDHANLS cells demonstrated significant growth advantage and colony-formation ability under continuous oxidative stress compared with vector control and LDHANES cells (Fig. 6a–c and Supplementary Fig. 12a). Next, we examined the expression of antioxidant genes and Wnt target genes in LDHAWT, LDHANLS, and LDHANES cells in response to H2O2. On one hand, LDHANLS cells presented higher expression levels of these genes under normal condition. On the other hand, LDHAWT but not LDHANES cells showed more pronounced target genes expression in response to H2O2 (Fig. 6d and Supplementary Fig. 12b).

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model with which direct cervical application of HPV16 E7-targeted transcription activator-like effector nucleases (TALENs) effectively mutated the E7 oncogene, reduced viral DNA load, and restored retinoblastoma-associated protein (RB1) function. Along with the TALEN applied, the cervical epithelium of the K14-HPV16 mouse showed a gradual loss of E7 and a reduction of epithelial proliferation. Meanwhile, the gradual loss of LDHA nuclear translocation correlated well with the decrease of H3K79 trimethylation levels in serial sections (Fig. 7e). Strikingly, on day 24 when E7 expression was almost undetectable, nuclear LDHA was eliminated and H3K79 trimethylation levels were dramatically attenuated (Fig. 7e). These in vivo data strongly support that HPV16 E7 induces LDHA nuclear translocation and H3K79 hypermethylation.

To further define the clinical relevance of our findings that HPV16 E7-induced LDHA nuclear translocation activated antioxidant response and Wnt signaling pathway, IHC analyses were performed to examine HPV16 E7 expression, LDHA nuclear localization, and H3K79 trimethylation levels in serial sections of 52 cases of human primary cervical cancer specimens. As shown in Fig. 7f and Supplementary Fig. 13, levels of HPV16 E7, nuclear LDHA expression, and H3K79 trimethylation were significantly positively correlated with each other, strongly supporting the crucial role of nuclear LDHA in HPV-positive cervical cancer.
Discussion

LDHA is implicated in tumorigenesis and tumor development. Loss of LDHA resulted in an elevated ROS production and a concomitant decrease in cell proliferation and invasion. Here, we have demonstrated a striking and previously unknown mechanism that nuclear LDHA senses ROS and gains a new catalytic function to promote HPV-induced cervical carcinogenesis: in response to HPV infection or oxidative stress, LDHA senses excessive ROS with a tetramer-to-dimer transition and nuclear translocation. Nuclear LDHA gains a noncanonical enzyme activity to produce an antioxidant metabolite, α-HB, which can protect cervical cancer cells from excessive oxidative stress and promote cell growth through epigenetic regulations. Interestingly, LDHA nuclear translocation appears to be essential for maintaining redox balance and sustaining cell proliferation.

LDHA’s nuclear localization has been observed in multiple cancer types, including colorectal cancer, breast cancer, prostate cancer, lung cancer, and liver cancer. (reference to Human Protein Atlas available from www.proteinatlas.org), but the cause of LDHA nuclear translocation and the function of nuclear LDHA remains unclear. Our study discovered a ROS-dependent manner of LDHA nuclear translocation in response to HR-HPV infection, and α-HB produced by nuclear LDHA act as an important antioxidant metabolite facilitating cancer development. Given the high demand for ATP as “fuel” and metabolic intermediates as “building blocks” in cancer cells, aberrant proliferation generally accompanied with enhanced ROS production, suggesting that the mechanism of LDHA nuclear translocation is potentially ubiquitous. Our study provides a clue for how cancer cell balancing the demand of high proliferation rate and the high production of ROS: LDHA acts as a sensor for overloaded ROS, and then produce α-HB in the nucleus to enhance antioxidant capacity.

Nuclear pore complexes (NPCs) tightly control protein shuttling between cytoplasmic and nuclear. Protein is required to associate with importins or exportins to enter or exit the nucleus. Importins bind their cargo with NLS amino acid sequence in the cytoplasm, then interact with NPCs and pass through its channel. However, no classic NLS sequences were found in LDHA, suggesting the existence of noncanonical NLS signal or some unknown binding partners of LDHA facilitating its nuclear translocation.

Upon oxidative stress, an increased flux of cysteine into glutathione synthesis has been well-studied. As a by-product of the methionine-to-glutathione pathway, α-HB production is directly linked to hepatic glutathione synthesis. Our findings showed that α-HB level arises upon HPV16/18 E7 induction and cellular ROS accumulation in cervical cancer cells. Moreover, we speculate that the accumulation of α-HB could further enhance antioxidant responses that may produce more α-HB, presenting a positive feed-forward loop. Similarly, β-hydroxybutyrate (β-HB) was found to suppress cellular oxidative stress through inhibition of histone deacetylase. We hypothesized if LDHA exerted the enzyme activity to catalyze β-ketobutyrate (β-KB) to β-HB. However, no such enzyme activity was detected under our tested experiment conditions. Recent studies also discovered another noncanonical enzyme activity of LDHA, catalyzing α-ketoglutarate (α-KG) to α-hydroxylglutarate (α-HG), under acidic pH conditions. However, the activity was not detectable under our tested experiment conditions.

We uncovered the α-HB accumulation increases H3K79 methylation levels, which is DOT1L-dependent. Furthermore, an interaction of DOT1L and LDHA has been identified upon α-HB treatment. However, how DOT1L methyl-transferase activity is regulated via its binding with LDHA remains unknown, which is worth further investigation.

In conclusion, we demonstrate that HPV16-induced nuclear translocation of LDHA is sufficient to trigger antioxidant responses and activate Wnt pathway, leading to cell survival and proliferation under oxidative stress. As such, blocking LDHA nuclear translocation may offer more opportunities to cervical cancer prevention and ROS-based cancer therapies.

Methods

Antibodies and reagents. Antibodies against LDHA (Cell Signaling Technology, 3582, with 1:2500 working dilution for western blot and 1:500 working dilution for IHC), GCLC (Cell Signaling Technology, 5719, with 1:1000 working dilution), α-actin (Invi- dilution), HPV16 E7 (Abcam, ab30731, with 1:2000 working dilution), Tubulin (Cell Signaling Technology, 3873, with 1:5000 working dilution), Lamin B1 (Cell Signaling Technology, 13435, with 1:2000 working dilution), H3K79me3 (Cell Signaling Technology, 4260, with 1:1000 working dilution), H3K79me2 (Cell Signaling Technology, 5427, with 1:1000 working dilution), H3K79me1 (Cell Signaling Technology, 12522, with 1:1000 working dilution), Histone H3 (Cell Signaling Technology, 4499, with 1:5000 working dilution), DOT1L (Abcam, ab72454, with 1:1000 working dilution), NRF2 (Abcam, ab62352, with 1:1000 working dilution), SOD1 (Abcam, ab31948, with 1:2000 working dilution), MYC (Abcam, ab32072, with 1:2000 working dilution), β-actin (Invitrogen, MAB-15739, with 1:2000 working dilution) were purchased commercially. Hydrogen peroxide solution (H$_2$O$_2$) (Sigma, S32381), N-acetyl cysteine (NAC) (Sigma, A7250), DAPI (Sigma, D9542), sodium pyruvate (Sigma, P5280), sodium (L) lactate solution (Sangon Biotech, A604046), sodium 2-ketobutyrate (Sigma, K0873), sodium 2-hydroxybutyrate (Santa Cruz, sc-258616), NADH (Sigma, N4505), EPZ004777 (Selleck, S7353), DPIC (Selleck, S6839), 50% glutaraldehyde solution (Sangon Biotech, A600875), and ML385 (MCE, HY-100523) were commercially obtained.
Cell culture and treatment. All the cell lines were purchased from American Type Culture Collection. HaCaT, HEK293T, SiHa, C33A, and HeLa cells were cultured in Dulbecco’s Modified Eagle’s Medium (DMEM) (Gibco), and HT-3 and U2OS cells were cultured in RPMI-1640 medium (Gibco) supplemented with 10% fetal bovine serum (Gibco) in the presence of penicillin, streptomycin (Gibco) at 37 °C in humidified atmosphere containing 5% CO2.

Primary human cervix keratinocytes (PHKs) were isolated from normal cervical epithelial obtained from iCell (HUM-iCell-f016). PHKs were cultured in EpiLife Medium (Thermo Fisher, MEPES009CA) with the addition of EpiLife Defined Growth Supplement (EDGS) (Thermo Fisher, S0125). The Coating Matrix (Thermo Fisher, R011K) was used to enhance the attachment, growth, and population doubling potential of human keratinocytes. For all the experiments, keratinocytes cultured between the third and fifth passages were used.
Fig. 6 Nuclear α-HB produced α-HB protects cervical cancer cells from oxidative stress. a–b, Nuclear LDHA promotes cell growth under oxidative stress. Growth curve of HeLa stable cells with oxidative stress. Colony-formation assay of HeLa stable cells treated with or without 10 μM H2O2 treatment. c–d, Nuclear LDHA increases colony formation under oxidative stress. Colony-formation assay of HeLa stable cells treated with or without 10 μM H2O2 treatment for 30 min, with or without the ROS-sensitive fluorescent dye CM-H2DCFDA by flow cytometry. a–b, α-HB balances cellular ROS level in LDHANES stable cells under oxidative stress. Cellular ROS were measured in HeLa LDHANES stable cells treated with 1 mM sodium α-HB supplement for 24 h. c–d, α-HB rescues LDHANES stable cells under oxidative stress. Growth curve of HeLa LDHANES stable cells treated with 10 μM H2O2, with or without different dose of sodium α-hydroxybutyrate supplement. e, α-HB rescues LDHANES colony formation under oxidative stress. Colony-formation assay of HeLa LDHANES stable cells treated with 10 μM H2O2, with or without different dose of sodium α-hydroxybutyrate supplement.

Sample preparation, iodoTMT labeling, and mass spectrometry. 5 × 10^6 cells cultured in media were washed in cold PBS, then lysed with lysis buffer (50 mM HEPES pH 7.9, 0.5 mM EDTA, 5 mM NaCl, 1% NP-40) for 10 min at 4 °C to pellet insoluble materials. The supernatant was collected into a new tube and incubate at 90 rpm in Thermomixer for 1 h at 37 °C protected from light. To remove the excessive iodoTMT-126 reagents, the proteins were precipitated with 200 μl of prechilled (-20 °C) acetone overnight at -20 °C. Precipitated proteins were washed twice with 500 μl of prechilled (-20 °C) acetone after centrifuged at 16,000 g for 10 min at 4 °C to pellet insoluble materials. The supernatant was collected into a new tube and incubated at 90 rpm in Thermomixer for 1 h at 37 °C protected from light. Then, the iodoTMT-126-labeled protein was precipitated by prechilled (-20 °C) acetone as described.

For protein digestion, the precipitated proteins were dissolved in 100 μl pH 7.0 ammonium bicarbonate buffer, pH 8.0. Add 2.5 μl of trypsin to sample (1/50: trypsin/protein, w/w). Digest the sample overnight at 37 °C water bath. After digestion, peptides were cleaned up using C18 tips and lyophilized. For iodoTMT reagent enrichment, anti-TMT resin was used according to the manufacturer’s protocol.

For LC-MS/MS analysis, purified peptides were resuspended in 1% acetonitrile and 1% formic acid (FA) in H2O and analyzed by LC (Ultimate 3000, Thermo Scientific) with packed capillary column (150 μm i.d. × 1 cm, 1.9 μm C18 reverse-phase fused-silica, Trap) using a 90 min nonlinear gradient at a flow rate of 600 nL/min. Eluted samples were analyzed by Q-Exactive HF (Thermo Scientific) equipped with self-packed capillary column (150 μm i.d. × 20 cm, 3 μm).

Immunoﬂuorescence and microscopy. Cells were plated at 1 × 10^5 per well on glass coverslips in 6-well culture plates and stimulated as described above. Cells were fixed with 4% paraformaldehyde for 15 min at room temperature, then rinsed twice in PBS. Permeabilization was performed with 0.2% Triton-X-100 in PBS for 30 min at room temperature. Primary antibody incubation was performed overnight in blocking buffer (0.5% BSA in PBS) at 4 °C. The following day, cells were washed twice in PBS, then incubated 4 h in secondary antibody in PBS at 4 °C. DAPI in PBS (5 mg/ml) was added prior to imaging. Microscopy was performed with OLYMPUS IX81 system. Image analysis was performed with the program CellSens Standard.

Generation of stable cell pools. To generate stable LDHA knockdown HeLa cell pools, shRNA targeting LDHA was constructed and targeting sequences were as follows:

| shLDHA-F | shLDHA-R |
|----------|----------|
| 5′-CCCGGCTACATACCTCTGCCATTGTCCGACGAGTAAAGCCGAGTGAGTCGTTTTCGATAT-3′ | 5′-AATTCAAAAAGCTACACATCCTGGGCTATTGCTCGAGCAATAATAGCGCCAGGATGTATGAGC-3′ |

The retrovirus was produced by using a two-plasmid packaging system as previously described. HeLa cells were infected with the retrovirus and selected with 2 μg/ml puromycin for 1 week. To generate LDHA knockdown and rescue stable cell pools, Flag-tagged human wild-type (WT), nuclear localization signal (NLS), or nuclear export signal (NES) LDHA was cloned into the retroviral pPCHIX vector and cotransfected with vectors expressing the gag and vsvg genes in HEK293T cells to produce retroviruses. HeLa cells stable LDHA knockdown were then infected, following a selection with 200 μg/ml hygromycin B for 1 week. The NLS and NES sequences were listed as follow: NLS: 5′-CCTAAGGAAAAGAGAAGAGATTTG-3′; NES: 5′-CTCTACGCTACACCGCCTTGGAGAAGCCTCACCTCTCT-3′.

Generation of knockout cells. CRISPR-Cas9-based gene knockdout was performed by oligonucleotides, three sgRNAs containing the LDHA-targeting sequences, and two sgRNAs containing NRF2-targeting sequences using lentiCRISPRv2 (Addgene) system. The targeting sequences were as follows:

| LDHA sgRNA1 | LDHA sgRNA2 |
|-------------|-------------|
| 5′-CATTAAAGATCATGTTAGGCGAC-3′ | 5′-CCGATTCTGGATCCTAAATG-3′ |
Measurement of intracellular and mitochondrial ROS levels. ROS and mitochondrial superoxide production were determined using a fluorescent dye chloromethyl-2′,7′-dichlorofluorescein diacetate (H$_2$DCF-DA, Sigma, 35845) and a specific mitochondrial superoxide indicator MitoSOX Red (Thermo Fisher, M36008), respectively. Briefly, cells with specified treatments were washed with PBS and incubated with 5 μM H$_2$DCF-DA (or 10 μM MitoSOX) at 37 °C for 30 min to load the fluorescent dye. Afterward, cells were washed twice with PBS, and trypsinized, centrifuged, resuspended in 500 μl PBS. Cells were kept in the dark until analysis by flow cytometry (Accuri C6, BD Biosciences).
Nuclear isolation. Isolation of nuclei was performed using the commercially available nuclei isolation kit: nuclei PURE prep from Sigma Aldrich (NUC201). Briefly, adherent cells were washed with PBS and scraped from the plate in the presence of lysis buffer. Cells (in lysis media) were carefully placed on top of a 1.8 M sucrose gradient and the resulting suspension was centrifuged at 30,000 g for 45 min in a precooled swing bucket ultracentrifuge. Nuclei were collected as a white pellet at the bottom of the centrifuge and washed with nuclei storage buffer (provided within the kit). Isolated nuclei were immediately subjected to metabolite quantification.

LDHA enzyme activity assay. The canonical and noncanonical LDHA enzyme activity was determined as described previously26. For endogenous, cells with specified treatments were lysed by NP-40 buffer (50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 0.3% Nonidet P-40) containing protease inhibitors [1 mg/ml aprotinin, 1 mg/ml leupeptin, 1 mg/ml pepstatin, 1 mM Na3VO4, and 1 mM phenylmethylsulfonyl fluoride (PMSF)], and for exogenous, Tag-flagged WT/LNS/NES LDHA proteins were overexpressed in cells, immunoprecipitated with Flag-beads, eluted by Flag peptides (Gison Biochemical), and subjected into activity assay with pyruvate and NADH as substrates for canonical enzyme activity and α-ketobutyrate (α-KB) and NADPH as substrates for noncanonical enzyme activity, respectively. Reaction mixture consists of 50 mM Tris-HCl (pH 7.4), 20 μM NADH, 2 μM pyruvate (or 3.3 mM α-KB) in a total volume of 500 μL. Reactions were initiated by adding the enzyme and analyzed at 25 °C. Activities were measured by the conversion of NADH to NAD+ which was monitored by measuring the decrease of fluorescence (Ex. 350 nm, Em. 470 nm, HITACH F-4600 fluorescence spectrophotometer) for NADH decomposition. LDHA enzyme activities were normalized to LDHA protein level.

Metabolite extraction. In total, 2 × 10⁶ cells or nuclei were resuspended in 800 µl of ice-cold 80% methanol and 20% ddH2O. Samples were vigorously vortexed and placed in liquid N2 for 10 min to freeze. Then thawed on ice for 10 min, before the freeze-thaw cycle was repeated. Samples were centrifuged at 13,000 rmp to pellet cell debris, lipids and proteins. The supernatant was evaporated and resulting metabolites were resuspended in HPLC-grade H2O. Metabolites were normalized to protein concentration.

Metabolite analysis by LC-MS/MS. The metabolite samples were resuspended in 20 µl HPLC-grade H2O for mass spectrometry. Three microliter of sample extract was injected and analyzed using a 6500 Q-Trap triple quadrupole mass spectrometer (AB Sciex) coupled to a Prominence HPLC System (Shimadzu) via multiple reaction monitoring (MRM). Metabolites were targeted in negative ion mode (IonSpray Voltage: −4500V). Ultimate AQ-C18 column (1.7 µm, 2 × 250 mm, Welch). The isolation of pyruvate, lactate, α-KB, and α-HB. Translocation column: (Superdex 200 Increase; GE Healthcare) was washed and equilibrated by cold PBS. Extracts were passed over the gel filtration column. The speed rate of flow is 0.4 ml/min. Fractions were collected every 0.25 ml per tube and analyzed by western blotting with LDHA antibody.

Gel filtration. Cells were treated with or without 10 μM H2O2 for 6 h and lysed by NP-40 buffer containing protease inhibitors (as above) for 30 min and centrifuged at 13,000 rpm to remove cell debris. The gel filtration was performed on a Superdex 200 Increase (GE Healthcare) and equilibrated by cold PBS. Extracts were run off the gel filtration column. The average band intensity of western blots was measured using Image J software. All the uncropped, full-size scans of western blots are presented in Supplementary Data 3.

Quantitative RT-PCR. Total RNA was isolated from cultured cells using Trizol reagent and reverse transcribed with random primers following the manufacturer’s instructions (TaKaRa). The cDNA was preceded to real-time PCR with gene-specific primers in the presence of SYBR Premix ExTaq (TaKaRa). PCR reactions were performed in triplicate on Roche LightCycler 480 System and the relative amount of cDNA was calculated by the comparative CT method using the β-actin as a control. Primer sequences were provided in Supplementary Data 3.

Chromatin immunoprecipitation (ChIP)-qPCR assays. ChIP-qPCR assays were performed as previously described28. Briefly, 2 × 10⁶ HaCaT, HT-3, and HeLa cells were crosslinked with 1% paraformaldehyde, lysed and sonicated using the Bioruptor at high-output power setting for 40 cycles (30 s ON and 30 s OFF). Solubilized chromatin was immunoprecipitated with ChIP-grade antibody for H3K79me2 or rabbit IgG (negative control), following that the antibodies were incubated overnight with A Sepharose beads overnight at 4 °C. Antibody-chromatin complexes were pulled down by protein A sepharose beads (Santa Cruz), washed with high salt buffer, LiCl buffer, TE buffer, and then eluted. After crosslink reversal in a water bath at 65 °C for 4 h and proteinase K digestion for 1 h at 55 °C, the CHIPed DNA was purified with QIAquick PCR Purification Kit (Qiagen, 28106). The DNA fragments were digested by QPCR, Histone H3K27 dimethylation marks were mapped at particular gene body regions of target genes (SOD1, CAT, CTNNB1, and MYC). Primers for the regions with peaks were designed by using the online primer program.
Cell proliferation assay and colony-formation assay. Cells were trypsinized, resuspended in PBS, and counted by using a hemocytometer. For cell proliferation assay, cells were plated in 6-well plates at 2500 cells per well in 2 ml media with or without H2O2, and plates were counted at 2, 4, 6, and 8 days. For colony-formation assay, cells were seeded in 6-well plates at 500 cells per well in 2 ml media with or without 10 μM H2O2. After 14 days, colonies were fixed in 4% paraformaldehyde and stained with 0.2% crystal violet. Colonies with >50 cells were counted. The media were exchanged every 24 h concerned about H2O2 decomposition.

Xenograft model. The procedures related to animal subjects were approved by ethic committee of the Institutes of Biomedical Sciences (IBS), Fudan University, Shanghai, China. In total, 5 × 106 HeLa LDHA KO cells with Vec/WT/NLS/NE5 were resuspended in PBS, and counted by using a hemocytometer. For cell proliferation assay and colony-formation assay, 2500 cells were per well in 2 ml media with or without 10 μM H2O2. After 14 days, colonies were fixed in 4% paraformaldehyde and stained with 0.2% crystal violet. Colonies with >50 cells were counted. The media were exchanged every 24 h concerned about H2O2 decomposition.

TALEN targeting HPV16 E7 in K14-HPV16 transgenic mice. K14-HPV16 transgenic mice have been described previously66. Breeding pairs of K14-HPV16 transgenic mice were provided by the National Cancer Institute (NCI) Mouse Repository (Frederick, MD, USA) [strain nomenclature: FVR-Cg-Tg (KRT14-HPV16 wt1Dh) and bred at the Experimental Animal Center, HUST. HPV16-positive female mice (6 to 8 weeks old) were randomly grouped. The DNA of TALEN plasmid was mixed with TurboFect In Vivo Transfection Reagent (RO541, Fermentas, Thermo Scientific) according to the manufacturer’s protocol. Mice were anesthetized with 3% pentobarbital sodium (intraperitoneal injection of 30 mg/kg mice body weight), and the polymer–DNA complexes were pipetted into the vagina using 200 μl pipet tips after washing with saline (in a maximum volume of 20 μl)66. Then, mice were kept in the dorsal position for at least 40 min under anesthesia to avoid loss of the complexes. The mice were euthanized after pentobarbital anesthetization at the indicated times. The vagina was dissected, fixed in 4% paraformaldehyde, and sectioned for IHC. The protocol for the assembly of the TALEN targeting HPV16 E7 was described previously66. The TALEN target sequence was as follows: 5′-ATGTTATAGTGGACGACAGGAGAATCAGTATTTCTGATTGCAATATAGACAGCTCGAGAGAGG-3′. Uppercase letters: left and right target cations (PRIDE) partner repository65 with the dataset identifier PXD011058. The datasets generated and analyzed during the current study are available within the article and the Supplementary Information files, and from the corresponding author upon reasonable request.

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35. Rosalki, S. B. & Wilkinson, J. H. Reduction of alpha-ketobutyrate by human serum. Nature 188, 1110–1111 (1960).

36. Sutendra, G. et al. A nuclear pyruvate dehydrogenase complex is important for the generation of acetyl-CoA and histone acetylation. Cell 158, 84–97 (2014).

37. Tsang, C. K., Liu, Y., Thomas, J., Zhang, Y. & Zheng, X. F. Superoxide dismutase 1 acts as a nuclear transcription factor to regulate oxidative stress resistance. Nat. Commun. 5, 3446 (2014).

38. Ghosh, R. et al. Allosteric inhibition of the IRE1α RNSase preserves cell viability and function during endoplasmic reticulum stress. Cell 158, 534–548 (2014).

39. Um, J. W. et al. Alzheimer amyloid-beta oligomer bound to postsynaptic prion protein activates Fyn to impair neurons. Nat. Neurosci. 15, 1227–1232 (2012).

40. Gu, X., Wang, H., Yang, J. J., Liu, X. & Liu, Z. R. Pyruvate kinase M2 regulates gene transcription by acting as a protein kinase. Mol. Cell. 45, 598–609 (2012).

41. Shi-y-Chang, N. et al. Influence of threonine metabolism on S-adenosylmethionine and histone methylation. Science 339, 222–226 (2013).

42. Mentch, S. J. et al. Histone methylation dynamics and gene regulation occur through the sensing of one-carbon metabolism. Cell Metab. 22, 861–873 (2015).

43. Daigle, S. R. et al. Selective killing of mixed lineage leukemia cells by a potent small-molecule DOT1L inhibitor. Cancer Cell. 20, 53–65 (2011).

44. Uren, A. et al. Activation of the canonical Wnt pathway during genital keratinocyte transformation: a model for cervical cancer progression. Cancer Res. 65, 6199–6206 (2005).

45. Ramachandran, I. et al. Linking H3K79 trimethylation to Wnt signaling through a novel Dot1-containing complex (DotCom). Genes Dev. 24, 574–589 (2010).

46. Ho, L. L. et al. DOT1L-mediated H3K79 methylation in chromatin is dispensable for Wnt pathway-specific and other intestinal epithelial functions. Mol. Cell. Biol. 33, 1735–1745 (2013).

47. Haddad, J. J. Antioxidant and prooxidant mechanisms in the regulation of redox(+) sensitive transcription factors. Cell Signal. 14, 879–897 (2002).

48. Singh, A. et al. Small molecule inhibitor of NFE2 selectively intervenes therapeutic resistance in KEAP1-deficient NSCLC tumors. ACS. Chem. Biol. 11, 3214–3225 (2016).

49. Hu, Z. et al. TALEN-mediated targeting of HPV oncogenes ameliorates HPV-related cervical malignancy. J. Clin. Invest. 125, 425–436 (2015).

50. Peng, M. et al. Aerobic glycolysis promotes T helper 1 cell differentiation through an epigenetic mechanism. Science 354, 481–484 (2016).

51. Zhao, D. et al. Lyssine-5 acetylation negatively regulates lactate dehydrogenase A and is decreased in pancreatic cancer. Cancer Cell. 23, 464–476 (2013).

52. Brand, A. et al. LDHA-associated lactic acid production blunts tumor immunosurveillance by T and NK Cells. Cell. Metab. 24, 857–867 (2016).

53. Arsenault, R. et al. Attenuation of LDHA expression in cancer cells leads to redox-dependent alterations in cytoskeletal structure and cell migration. Cancer Lett. 338, 255–266 (2013).

54. Jin, L. et al. Phosphorylation-mediated activation of LDHA promotes cancer cell invasion and tumour metastasis. Oncogene 36, 3797–3806 (2017).

55. Uhlen, M. et al. Proteomics. Tissue-based map of the human proteome. Science 347, 124619 (2015).

56. Cramer, S. L. et al. Systemic depletion of L-cyst(e)ine with cyst(e)inase increases reactive oxygen species and suppresses tumor growth. Nat. Med. 23, 120–127 (2017).

57. Lord, R. S. & Brailey, J. A. Clinical applications of urinary organic acids. Part I: detoxification markers. Altern. Med. Rev. 13, 265–215 (2008).

58. Shimazu, T. et al. Suppression of oxidative stress by beta-hydroxybutyrate, an endogenous histone deacetylase inhibitor. Science 339, 211–214 (2013).

59. Gao, X. et al. Acetate functions as an epigenetic metabolite to promote lipid synthesis under hypoxia. Nat. Commun. 7, 11960 (2016).

60. Wang, Y. P. et al. Arginine Methylation of MDH1 by CAR1 induces glutamine metabolism and suppresses pancreatic cancer. Mol. Cell 64, 673–687 (2016).

61. Plummer, D. T., Elliott, B. A., Cooke, K. B. & Wilkinson, J. H. Organ specificity and lactate-dehydrogenase activity. I. The relative activities with pyruvate and 2-oxobutyrate of electrophoretically separated fractions. Biochem. J. 87, 416–422 (1963).

62. Armit, J. M., Munger, K., Howley, P. M. & Hanahan, D. Progressive squamous epithelial neoplasia in K14-human papillomavirus type 16 transgenic mice. J. Virol. 68, 4358–4368 (1994).

63. Palliser, D. et al. An siRNA-based microbicidal protects mice from lethal herpes simplex virus 2 infection. Nature 439, 89–94 (2006).

64. Viscaino, J. A. et al. The proteomics identifications (PRIDE) database and associated tools: status in 2013. Nucleic Acids Res. 41, D1063–D1069 (2013).

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Author contributions

Y.L and J.Z.G performed the experiments, analyzed the data and wrote the paper. Y.L designed and performed histopathological experiments and analysis. K.W. aided in paper preparation. W.D. and H.W. conducted K14-HPV16 transgenic mouse trials. X.L., S.Z. and I.Z. collected human samples and informed consents. X.C.L performed the protein crosslink experiments. H.B.Y performed the enzymatic experiments and analyzed the data. C.X. assisted histopathological experiments and analysis. W.G. assisted schematic graph design and drawing. Y.P.W designed a part of the study and helped to interpret part data, W.H. helped to interpret part data, W.Y. supported the research. C.H. and Q.Y.L conceived the idea, designed and supervised the study, analyzed the data, and co-wrote the paper.

Additional information

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