Aldolase A promotes epithelial-mesenchymal transition to increase malignant potentials of cervical adenocarcinoma

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Abstract
Recent studies have revealed that metabolic reprogramming is closely associated with epithelial-mesenchymal transition (EMT) during cancer progression. Aldolase A (ALDOA) is a key glycolytic enzyme that is highly expressed in several types of cancer. In this study, we found that ALDOA is highly expressed in uterine cervical adenocarcinoma and that high ALDOA expression promotes EMT to increase malignant potentials, such as metastasis and invasiveness, in cervical adenocarcinoma cells. In human surgical specimens, ALDOA was highly expressed in cervical adenocarcinoma and high ALDOA expression was correlated with lymph node metastasis, lymphovascular infiltration, and short overall survival. Suppression of ALDOA expression significantly reduced cell growth, migration, and invasiveness of cervical cancer cells. Aldolase A expression was partially regulated by hypoxia-inducible factor-1α (HIF-1α). Shotgun proteome analysis revealed that cell-cell adhesion-related proteins were significantly increased in ALDOA-overexpressing cells. Interestingly, overexpression of ALDOA caused severe morphological changes, including a cuboidal-to-spindle shape shift and reduced microvilli formation, coincident with modulation of the expression of typical EMT-related proteins. Overexpression of ALDOA increased migration and invasion in vitro. Furthermore, overexpression of ALDOA induced HIF-1α, suggesting a positive feedback loop between ALDOA and HIF-1α. In conclusion, ALDOA is overexpressed in cervical adenocarcinoma and contributes to malignant potentials of tumor cells through modulation of HIF-1α signaling. The feedback loop between ALDOA and HIF-1α could become a therapeutic target to improve the prognosis of this malignancy.

KEYWORDS
aldolase A, cervical adenocarcinoma, epithelial-mesenchymal transition, hypoxia-inducible factor-1α, metabolic reprogramming
1 | INTRODUCTION

The incidence of uterine cervical adenocarcinoma has been dramatically increasing worldwide, predominantly in young women, despite the fact that the incidence of squamous cell carcinoma (SCC) has been decreasing. It has been shown that adenocarcinoma has a worse prognosis than that of SCC at the same stage and with the same tumor size. The main reasons for the worse prognosis are a higher rate of metastases and resistance to chemoradiotherapy. Consequently, a novel therapeutic strategy is needed to improve the outcome of cervical adenocarcinoma.

Fructose-bisphosphate aldase is one of the key glycolytic enzymes that catalyzes the reversible reaction of fructose-1,6-bisphosphate to glyceraldehyde-3-phosphate and dihydroxyacetone phosphate. Aldolase is ubiquitously distributed in all organs and/or cells and it plays an essential role in ATP biosynthesis, especially in a glycolytic pathway under anaerobic conditions.

Aldolase A (ALDOA) is overexpressed in various cancers including squamous cell lung cancer, hepatocellular carcinoma, colonic cancer, osteosarcoma, and pancreatic cancer. In recent years, some researchers have reported that overexpression of ALDOA is involved in malignant behaviors of various cancer cells. However, there is no information about the relationship between expression of ALDOA and malignant behavior of cervical adenocarcinoma cells.

In this study, we examined the expression of ALDOA in cervical adenocarcinoma tissues by immunohistochemistry and the relationships between ALDOA expression and clinicopathologic features. We also investigated the importance of ALDOA expression in the malignant behavior of uterine cervical cancer cells. This is the first report showing that ALDOA is overexpressed in cervical adenocarcinoma specimens and that overexpression of ALDOA enhances malignant behavior through induction of epithelial-mesenchymal transition (EMT).

2 | MATERIALS AND METHODS

2.1 | Immunohistochemistry and immunohistochemical analysis of surgical specimens

Specimens of 53 cases of cervical adenocarcinoma including adenocarcinoma in situ (AIS) obtained by surgical resection during the period from 2004 to 2012 were retrieved from the pathology file of Sapporo Medical University Hospital (Sapporo, Japan). This study was approved by the Institutional Review Board of Sapporo Medical University (IRB study number 302-197). Written informed consent was obtained from each patient who participated in the investigation. As controls, adjacent nonneoplastic regions were examined as normal tissues (n = 40). Clinicopathologic features of the patients were described previously. Immunohistochemistry was carried out with anti-ALDOA (1:500; Sigma-Aldrich) as described previously.

Surgical specimen staining patterns were scored as follows: score 0, no reactivity or cytosolic reactivity in less than 10% of tumor cells; score 1+, faint/almost no cytosolic reactivity in 10% or more of tumor cells; score 2+, weak to moderate cytosolic reactivity in 10% or more tumor cells; and score 3+, moderate to strong cytosolic reactivity in 10% or more of tumor cells. For statistical purposes, samples with scores 0 and 1+ were considered negative, and those with scores 2+ and 3+ were considered positive. When evaluating the slides, the observers (YS, MM, and AT) were blinded to the clinical data. Discordant cases were discussed, and a consensus was reached.

2.2 | Cell culture and treatment

The human cervical adenocarcinoma cell lines Hela229 and OMC4 were purchased from RIKEN Bio-Resource Center (Tsukuba, Japan) and HCA1 was from JCRB Cell Bank (Osaka, Japan). The human cervical adenocarcinoma cell line CAC-1 and TMCC-1 were provided by our colleague Dr Hayakawa. Cells were maintained as described previously. Human ALDOA-specific siRNAs (5′-GUGUCAUCCCUUCCAUGA-3′ and 5′-GUAUCUCUCCUCCAUGA-3′), human hypoxia-inducible factor-1α-specific siRNAs (5′-GAUAAACGUGUUGAGUGU-3′ and 5′-GAUUAACGUGUUGAGUGU-3′) and siRNA universal negative control were purchased from Sigma-Aldrich.

Transfection of siRNA was carried out by using RNAiMAX Transfection Reagent (Thermo Fisher Scientific) according to the manufacturer’s instructions. The coding sequence of human ALDOA gene was obtained by RT-PCR by using total RNA of HCA1 cells as a template and using primers 5′-CATAACGGCTCTACAAATGCACTG-3′ and 5′-CGGAATCTTATAGGCGTGGTTAGAGA-3′. The amplified fragment was cloned between the HindIII site and EcoRI site of pCMV10-3xFLAG vector (Thermo Fisher Scientific). To obtain stable clones overexpressing ALDOA, the 3xFLAG-ALDOA expressing vector was transfected in HCA-1 cells by using Lipofectamine 3000 (Thermo Fisher Scientific) followed by selection with 800 μg/ml neomycin for 14 days. The neomycin-resistant cells were then seeded in 96-well plates at a density of 1 cell/well to obtain single clones. The ALDOA-overexpressing cells (FLAG-ALDOA cells) were screened by western blot by using anti-FLAG (M2) Ab. For hypoxia incubation, cells were maintained at 2% O2, 5% CO2, and 93% N2 at 37°C, using an automatic multigas incubator (IncuSafe, MCO-SC-P; PHC). Primary Ab information is listed in Table S1.

2.3 | Cellular ATP detection assay

A luminescence ATP detection assay system, ATPlite 1step (Perkin Elmer, Boston, MA), was used to measure the level of cellular ATP according to the manufacturer’s instructions. Briefly, subconfluent cells (6-cm dish) were trypsinized and seeded in a white-wall, 96-well plate (Corning) at a concentration of 103 cells/100 μl medium per well. ATPlite 1step reagent (100 μl) was added and then the luminescence was measured on
2.4 | Cell proliferation assay

In the WST-8 assay, cells were seeded in 96-well plates and their viability was assessed at 24 hours after incubation by using a CCK-8 (Dojindo Laboratories) according to the manufacturer’s instructions. Absorbance at a wavelength of 450 nm was measured by using the Varioskan LUX multimode microplate reader (Thermo Fischer Scientific). Colony forming assay was carried out as described previously. Each experiment was independently repeated 3 times.

2.5 | Immunocytochemistry of cell blocks and immunofluorescence microscopy

Cell blocks were prepared by using the sodium alginate method as described previously.

2.6 | Cell migration and invasion assays

The migration assay was carried out using Transwell (Corning; 8-μm pore polycarbonate membrane insert) in 24-well dishes and the invasion assay was performed using Biocoat Matrigel (Corning; pore size, 8-μm) as described previously. Each experiment was independently repeated 3 times.

2.7 | Peptide preparation

Protein was extracted using a phase transfer surfactant method as described previously. Briefly, cells were lysed with 50 mmol/L ammonium bicarbonate containing 12 mmol/L sodium deoxycholate, 12 mmol/L sodium N-lauroylsarcosinate, and EDTA-free protease inhibitor cocktail (Roche Diagnostics). Lysates were heated at 95°C for 5 minutes, sonicated, and then centrifuged at 19 000 g for 15 minutes at room temperature. Supernatants were collected and protein concentration was measured by a reducing agent compatible version of the Pierce microplate BCA protein assay kit (Thermo Fischer Scientific). Fifty micrograms of protein lysate was reduced with 10 mmol/L DTT, alkylated with 20 mmol/L.

![Figure 1](image)

**Figure 1** Aldolase A (ALDOA) is highly expressed in surgical specimens of cervical neoplasms. A-D, Immunohistochemistry of ALDOA in surgical specimens of human cervical adenocarcinoma. ALDOA was strongly expressed in adenocarcinoma (ADC), whereas it was undetectable in the nonneoplastic cervical epithelium (CE). Scale bar = 50 μm. E, Immunoreactive intensities of ALDOA in CE and ADC. F, G, Kaplan-Meier estimates of overall survival (OS) and relapse-free survival (RFS) of patients with cervical adenocarcinoma. High expression level of ALDOA was significantly correlated with worse OS ($P = .0362$).
iodoacetamide, and then diluted with 50 mmol/L ammonium bicarbonate, followed by digestion with 1:50 (w/w) trypsin for 18 hours at 37°C. After digestion, an equal volume of ethyl acetate containing 1% TFA was added. The mixture was vortexed for 1 minute and centrifuged at 15 600 g for 3 minutes. The lower phase was discarded. The upper aqueous phase was concentrated under vacuum and then desalted by GL-Tip SDB according to the instructions of the manufacturer (GL Science).

2.8 | Proteome analysis

Samples were dissolved in 0.1% formic acid and loaded into a nanoflow UHPLC (Easy-nLC 1000 system; Thermo Fisher Scientific) online-coupled to an Orbitrap mass spectrometer equipped with a nanospray ion source (Q-Exacte Plus; Thermo Fisher Scientific). Samples were separated by using a 75 µm × 20 cm capillary column with a particle size of 3 µm (NTCC-360; Nikkyo Technos) by applying a linear gradient ranging from 5% to 35% buffer B (100% acetonitrile and 0.1% formic acid) at a flow rate of 300 nL/min for 120 minutes. In mass spectrometry analysis, survey scan spectra were acquired at a resolution of 70 000 at 200 m/z with a target value of 3e6 ions, ranging from 350 to 2000 m/z with charge states between 1+ and 4+. We applied a data-dependent top 10 method that generates high-energy collision dissociation fragments for the 10 most intense precursor ions per survey scan. The tandem mass spectrometry (MS/MS) resolution was 17 500 at 200 m/z with a target value of 1e5 ions.

For MS/MS data analysis, we used the Sequest HT (Thermo Fisher Scientific) and Mascot version 2.5 (Matrix Science) algorithms embedded in the Proteome Discoverer 2.2 platform (Thermo Fisher Scientific), and the peak lists were searched against the UniProt human databases. The tolerance of precursor ions and fragment ions were set to 10 ppm and 0.02 Da, respectively.

2.9 | Scanning electron microscopy

For scanning electron microscopy, cells grown on coverslips were fixed with 2.5% glutaraldehyde in PBS overnight at 4°C. After several rinses with PBS, the cells were postfixed in 1% OsO₄ in PBS at 4°C for 3 hours and washed with distilled water, followed by dehydration through a graded series of ethanol and freeze drying. Samples were sputter-coated with platinum and examined under a scanning electron microscope (S4300; HITACHI) operating at 10 kV.

2.10 | Statistical analysis

The measured values are presented as mean ± SD. Data were analyzed and compared using the unpaired 2-tailed Student’s t test, Fisher’s exact test, and Kruskal-Wallis test. Survival rates were calculated by the Kaplan-Meier method and compared by the log-rank test. Statistical significance was accepted when P < .05 (**P < .01). All statistical analyses were undertaken with EZR software.

3 | RESULTS

3.1 | Expression profiles of ALDOA in surgical specimens of cervical adenocarcinoma

First, we undertook the immunohistochemistry of surgical specimens for ALDOA in uterine cervical adenocarcinomas. In nonneoplastic cervical gland tissues, immunostaining of ALDOA was faint or absent (Figure 1A,C). In contrast, strong and diffuse immunostaining of ALDOA was observed in the cytoplasm in adenocarcinoma (ADC) (Figure 1B,D). On the basis of the immunoreactive intensity, the patients were classified into 2 groups: a high ALDOA expression group (intensity of 2+ or 3+) and a low ALDOA expression group (intensity of 1+ or 0). Twenty-four (55.9%) of 43 ADC cases had high ALDOA expression. Those cases included 18 cases (41.9%) and 6 cases (14.0%) with intensities of 3+ and 2+, respectively. Five (50.0%) of 10 AIS cases had high ALDOA expression. They included 2 cases (20.0%) and 3 cases (30.0%) with intensities of 3+ and 2+, respectively.

| TABLE 1 | Clinicopathologic parameters of cervical adenocarcinoma |
|----------------------------------------|----------------------|----------------------|
| Diameter (mm)  | ALDOA | N | P value |
| <=40  | 41 | 21 | 20 | .3460 |
| >40  | 12 | 3 | 9 |
| UICC  |  |  |  |  |
| 0  | 10 | 5 | 5 | .1050 |
| I  | 32 | 17 | 15 |
| II  | 5 | 2 | 3 |
| III  | 6 | 0 | 6 |
| Tumor factor  |  |  |  |  |
| pT0  | 10 | 5 | 5 | .3710 |
| pT1  | 33 | 17 | 16 |
| pT2  | 9 | 2 | 7 |
| pT3  | 1 | 0 | 1 |
| N factor  |  |  |  |  |
| N0  | 47 | 24 | 23 | .0266 |
| N1  | 6 | 0 | 6 |
| Lymphovascular infiltration  |  |  |  |  |
| Negative  | 38 | 22 | 16 | .0051 |
| Positive  | 15 | 2 | 13 |

ALDOA, aldolase A.
respectively. Thus, ALDOA expression was high in 29 (54.7%) of the 53 AIS/ADC cases, including 20 cases (37.7%) with an intensity of 3+ and 9 cases (17.0%) with an intensity of 2+ (Figure 1E).

3.2 | Correlations between expression of ALDOA and clinicopathologic features

We next evaluated the relationships of ALDOA expression intensity with clinicopathologic features and outcome of cervical adenocarcinoma. As shown in Table 1, high ALDOA expression was significantly correlated with lymph node metastasis (\( P = .0266 \)) and lymphovascular infiltration (\( P = .0051 \)). There was no significant correlation between ALDOA expression and histological type, diameter, UICC stage, or tumor factor. The relationships between ALDOA expression and relapse-free survival and overall survival of patients with cervical adenocarcinoma were assessed by using the Kaplan-Meier method. Overall survival was significantly shorter in patients with high ALDOA expression than in patients with low ALDOA expression (Figure 1F), but there was no correlation between ALDOA expression and relapse-free survival (Figure 1G). These results suggest that ALDOA is not only highly expressed in cervical adenocarcinoma but also might play a role in the development of cervical adenocarcinoma.

3.3 | Knockdown of ALDOA attenuates the malignant potential of cervical adenocarcinoma cells

To examine the effects of ALDOA expression on tumor cells, we investigated the expression patterns of ALDOA in human uterine cervical cancer cell lines HCA1, Hela229, TMCC1, and CAC1. In all of the cell lines examined, ALDOA was constitutively expressed (Figure 2A). Among the cell lines, the well-differentiated type of cervical adenocarcinoma cell line HCA1 was mainly used for subsequent experiments because the cell line retains some epithelial properties, including morphologically intact adherence junctions and tight junctions, and does not carry human herpes viruses.14

First, we suppressed the expression of ALDOA by ALDOA-specific siRNAs (Figures 2B, S1A and S2). In a WST-8 cell proliferation assay, the percentage of proliferative cells in ALDOA knockdown (KD) cells was significantly lower than that in control cells treated with scrambled siRNA (Figures 2C and S1B). In a

![Figure 2](https://example.com/fig2.png)

**FIGURE 2** Knockdown (KD) of aldolase A (ALDOA) inhibits proliferation, migration, and invasion of cervical adenocarcinoma cells. A, ALDOA was expressed in all of the tested cervical cancer cell lines. B, Western blot analysis. Knockdown of ALDOA expression was achieved by ALDOA-specific siRNAs compared to scrambled siRNA (scr) in HCA1 cells. C, D, ALDOA siRNAs significantly reduced the proliferation ability of HCA1 cells in WST-8 (C) and colony formation (D) assays. E, Immunohistochemistry of anti-cleaved caspase-3 (apoptosis marker) Ab in cell block samples. F, Transwell migration assay. ALDOA-KD significantly inhibited migration of HCA1 cells. G, Matrigel invasion assay. ALDOA-KD significantly inhibited invasion of HCA1 cells.
colony formation assay, the number of colonies was significantly smaller for ALDOA-KD cells than for control cells (Figures 2D and S1C). In addition, a large number of cleaved caspase-3-positive cells, an indicator of apoptosis, was observed for ALDOA-KD cells compared with control cells (Figure 2E). These results indicated that ALDOA-KD inhibits cell proliferation, partially due to increased apoptosis, in cervical adenocarcinoma cells. In a migration assay using a Transwell membrane, the number of ALDOA-KD cells migrating through the membrane was significantly smaller than that of control cells (Figures 2F and S1D). Also, in an invasion assay using a Matrigel matrix-coated chamber, the number of ALDOA-KD cells penetrating through the matrix was significantly smaller than that of control cells (Figure 2G). These results indicate that ALDOA expression contributes to the malignant potential of cervical adenocarcinoma cells.

### 3.4 Aldolase A is induced by hypoxic conditions in cervical adenocarcinoma cells

Next, we examined the factor responsible for increased expression of ALDOA. We hypothesized that ALDOA expression might be induced by hypoxia, as aldolase is well known to be involved in the anaerobic glycolytic pathway. As expected, ALDOA expression was induced together with expression of HIF-1α at 24 hours after culturing cells under hypoxic conditions (2% O₂) (Figure 3A). Under hypoxic conditions, KD of HIF-1α inhibited induction of both HIF-1α and ALDOA (Figure 3C), whereas knockdown of ALDOA inhibited ALDOA expression but did not affect HIF-1α induction (D) under hypoxic conditions. Expression of proliferating cell nuclear antigen (PCNA) was induced by ALDOA overexpression. The amount of intracellular ATP was significantly larger in ALDOA-overexpressing HCA1 cells than in control cells. ALDOA overexpression significantly promoted the proliferation ability of HCA1 cells in WST-8 (G) and a colony formation (H) assays. A-E, Western blot analysis.
manner (Figure 3B). These results suggest that hypoxic conditions might be associated with increased ALDOA expression through HIF-1α in cervical adenocarcinoma cells.

### 3.5 Overexpression of ALDOA enhances the proliferation of cervical adenocarcinoma cells

To further analyze the effect of ALDOA overexpression, we established stable clones expressing 3xFLAG-tagged ALDOA, hereinafter referred to as FLAG-ALDOA cells (Figure 3E). As expected from the role of ALDOA, the amount of cellular ATP was significantly larger in FLAG-ALDOA cells than in control cells (Figure 3F), indicating that ATP synthesis was increased by ALDOA overexpression. In western blot analysis, these clones showed enhancement of the expression of proliferating cell nuclear antigen, an indicator of cell proliferation (Figure 3E). In a WST-8 cell proliferation assay, overexpression of ALDOA significantly enhanced the proliferation of HCA1 cells (Figure 3G). In a colony formation assay, the number of colonies was significantly larger in FLAG-ALDOA cells than in control cells (Figure 3H). These results indicate that ALDOA overexpression enhances the proliferation of cervical adenocarcinoma cells.

### 3.6 Association between ALDOA overexpression and downregulation of cell-cell adhesion molecules was revealed by proteomic analysis

To clarify the role of ALDOA overexpression, we undertook comparative shotgun proteomic analyses. Protein samples from FLAG-ALDOA cells and control cells were digested by trypsin, and the digested peptides were subjected to MS. A label-free quantitation method was used to compare protein expression patterns between FLAG-ALDOA cells and control cells. To ensure the quality and precision of data, each sample measurement was independently repeated...
A total of 1775 unique proteins were identified (Data S1); of these, 172 proteins were upregulated (ratio > 1.5, \( P < .05 \); Table S2) and 172 proteins were downregulated in FLAG-ALDOA cells (ratio < 0.67, \( P < .05 \); Figure 4A,B and Data S1). Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes pathway analyses revealed that enriched GO terms for downregulated proteins were predominantly associated with cell-cell adhesion terms including cell-cell adhesion (GO:0098609), actin cytoskeleton reorganization (GO:0031532), cell-cell adherens junction (GO:0005913), focal adhesion (GO:0005925), and cadherin binding involved in cell-cell adhesion (GO:0098641) (Figure 4C and Data S2). As shown in Data S2, adherens junction molecules (\( \alpha \)-catenin and \( \beta \)-catenin) and a tight junction molecule (junctional adhesion molecule-A [JAM-A]) were included in the list of downregulated proteins in FLAG-ALDOA cells. Listed proteins in each GO term are shown in Data S2. Enriched GO terms for upregulated proteins are shown in Table S2. The results suggest that ALDOA overexpression could influence cell-cell adhesion of cervical adenocarcinoma cells.

3.7 Aldolase A overexpression leads to EMT-like morphological alteration in cervical adenocarcinoma cells

The results of proteomic analysis indicated that ALDOA overexpression causes a comprehensive change in cell-cell adhesion-associated proteins, evoking EMT. To determine whether ALDOA overexpression is associated with such a phenomenon, we undertook cell-based assays to assess the characteristics of EMT. Phase-contrast microscopy revealed that overexpression of ALDOA (ALDOA-1 and ALDOA-2) caused morphological changes from an oval-cuboidal shape into a spindle shape, resembling a mesenchymal cell-like shape (Figure 5A-C). In immunofluorescence, immunoreactivity of E-cadherin, one of the most important molecules of cell-cell adhesion, was significantly suppressed by ALDOA overexpression. Phalloidin staining (red) shows that stress fiber formation was induced by ALDOA overexpression. G. Scanning electron microscopy. The formation of microvilli was suppressed by ALDOA overexpression. Scale bar = 10 \( \mu \)m (C, F), 5 \( \mu \)m (G). ctrl, control.
in control cells (Figure 6B). In an invasion assay using a Matrigel matrix-coated chamber, the number of cells invading through the matrix was significantly larger in FLAG-ALDOA cells than in control cells (Figure 6C). Of note, levels of CD44 and aldehyde dehydrogenase 1 (ALDH1) were increased in FLAG-ALDOA cells (Figure 6A).

**FIGURE 6** Aldolase A (ALDOA) overexpression leads to epithelial-mesenchymal transition (EMT)-like alterations in cervical adenocarcinoma cells. A, ALDOA overexpression suppressed the expression of cell-cell adhesion-associated proteins (α-catenin, β-catenin, junctional adhesion molecule-A [JAM-A], and E-cadherin) and increased the expression of EMT regulatory proteins (Snail, Slug, and hypoxia-inducible factor-1α [HIF-1α]) in HCA1 cells. Western blot analysis. B, C, Transwell migration assay and Matrigel invasion assay. ALDOA overexpression significantly promoted migration (B) and invasion (C) of HCA1 cells. D, Illustration showing that ALDOA and HIF-1α form a positive feedback loop to promote malignant potentials of cervical adenocarcinoma cells. ctrl, control

4 | DISCUSSION

This is the first study showing that a high level of ALDOA expression plays a significant role in cervical adenocarcinoma. An immunohistochemical study clearly showed that high ALDOA expression was correlated with some clinicopathologic features, including lymphovascular infiltration and lymph node metastasis, and with poor prognosis (Table 1 and Figure 1). These results suggest that ALDOA is not just highly expressed in cervical adenocarcinoma but might play roles in disease progression, as suggested by the results of cell-based assays.

As shown in Figure 2, knockdown of ALDOA significantly suppressed proliferation, migration, and invasiveness of cervical adenocarcinoma cells, indicating that ALDOA expression contributes to malignant potentials of cervical adenocarcinoma cells. Previous studies showed that ALDOA is highly expressed in malignant neoplasia of various organs and is involved in malignant behaviors of cancer cells.19-25,38 Cervical adenocarcinoma is now included in the list of malignancies with high ALDOA expression, and our results indicate
that ALDOA is a potential biomarker and a potential therapeutic target of cervical adenocarcinoma.

Interestingly, our cell biological experiments revealed that overexpression of ALDOA caused drastic morphological changes in cervical adenocarcinoma cells (Figure 5). The observed cuboidal-to-spindle morphological change and loss of microvilli are typical features of EMT cells.59 The ALDOA-induced morphological changes were accompanied by suppression of the expression of adherens and tight junction proteins including E-cadherin, β-catulin, and JAM-A (Figure 6), which has also been shown to occur during the EMT process.40 As described above, ALDOA has been shown to be highly expressed in malignant neoplasia of various organs and to be involved in malignant behaviors of cancer cells; however, the underlying molecular mechanisms have not been fully elucidated.41,42 We found that overexpression of ALDOA induced the expression of HIF-1α, which is known to modulate EMT through regulation of EMT-related transcription factors including Twist, Snail, Slug, Smad interacting protein 1 (Sip1), and zinc finger E-box-binding homeobox 1 (ZEB1).43-46 In this study, we also confirmed that some of the transcription factors were increased by ALDOA overexpression (Figure 6) and they are probably responsible for downregulation of adherens and tight junction proteins (Figure 6). Our results clearly showed that overexpression of ALDOA could induce EMT in cervical adenocarcinoma cells. ALDOA-induced EMT may explain the positive correlations of high ALDOA expression with lymph node metastasis and lymphovascular infiltration in surgical specimens.

Of note, we observed that a hypoxic condition, a representative factor activating HIF-1α, induced ALDOA expression in cervical adenocarcinoma cells (Figure 3). Together with the results showing that ALDOA induced HIF-1α expression (Figure 6), we propose that ALDOA and HIF-1α form a positive feedback loop to promote EMT-related tumor progression in cervical adenocarcinoma (Figure 6). In this study, we also found that overexpression of ALDOA induced the expression of CD44 and ALDH1 (Figure 6). A future study should be carried out to determine whether ALDOA overexpression leads to the emergence of a stem cell-like property through induction of EMT as induction of CD44 and ALDH1 might reflect the acquisition of cancer stemness.47

Metabolic reprogramming, a concept that has emerged in the past few decades, refers to cellular adaptation mechanisms by comprehensive alteration in lipid, glutamine, and sugar metabolism in cancer cells.45,48 Aldolase A is one of the major glycolytic enzymes, and overexpression of ALDOA could play crucial roles in metabolic reprogramming of cervical adenocarcinoma because the process of metabolic reprogramming is frequently triggered by increased expression and/or activity of metabolism-related enzymes.49 How metabolic pathways are influenced by ALDOA overexpression in cervical adenocarcinoma should be examined in future studies. The results of such studies would contribute to the establishment of therapeutic strategies for high ALDOA expression-related cancers, including cervical adenocarcinoma.

In conclusion, ALDOA is overexpressed in cervical adenocarcinoma and contributes to malignant potentials of tumor cells through modulation of HIF-1α signaling. The feedback loop between ALDOA and HIF-1α could become a therapeutic target to improve the prognosis of this malignancy.

ACKNOWLEDGMENTS

The authors would like to thank Yui Kawami and Taro Murakami for technical assistance with the experiments. This work was supported by JSPS KAKENHI Grant Numbers JP17KO8697, JP17KO8698, JP18K15084, and JP19K16561 and Grants from the Suhara Foundation.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Saito Y, Takasawa A, Takasawa K, et al. Aldolase A promotes epithelial-mesenchymal transition to increase malignant potentials of cervical adenocarcinoma. Cancer Sci. 2020;111:3071–3081. https://doi.org/10.1111/cas.14524