RESEARCH COMMUNICATION

Expression and Functional Role of ALDH1 in Cervical Carcinoma Cells

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Abstract

Tumor formation and growth is dictated by a very small number of tumor cells, called cancer stem cells, which are capable of self-renewal. The genesis of cancer stem cells and their resistance to conventional chemotherapy and radiotherapy via mechanisms such as multidrug resistance, quiescence, enhanced DNA repair abilities and anti-apoptotic mechanisms, make it imperative to develop methods to identify and use these cells as diagnostic or therapeutic targets. Aldehyde dehydrogenase 1 (ALDH1) is used as a cancer stem cell marker. In this study, we evaluated ALDH1 expression in CaSki, HeLa and SiHa cervical cancer cells using the Aldefluor method to isolate ALDH1-positive cells. We showed that higher ALDH1 expression correlated with significantly higher rates of cell proliferation, microsphere formation and migration. We also could demonstrate that SiHa-ALDH1-positive cells were significantly more tumorigenic compared to SiHa-ALDH1-negative cells. Similarly, SiHa cells overexpressing ALDH1 were significantly more tumorigenic and showed higher rates of cell proliferation and migration compared to SiHa cells where ALDH1 expression was knocked down using a lentivirus vector. Our data suggested that ALDH1 is a marker of cervical cancer stem cells and expand our understanding of its functional role.

Keywords: Cervical cancer - adhehyde dehydrogenase - cancer stem cells - proliferation - migration - tumorigenic

Introduction

Cervical cancer is the most commonly occurring cancer of the female reproductive tract (Stanley, 2010). Human papilloma virus (HPV) infection has been shown to be a necessary cause of cervical cancer and is implicated in the pathogenesis of 99.7% of all cervical cancer cases (Walboomers et al., 1999). HPV infection often occurs at the junction of the cervical squamous epithelium and columnar epithelium (the transition zone) A majority (85-90%) of cervical cancers are squamous cell carcinomas and 10-15% are adenocarcinomas (Moody and Laimins, 2010). Early stage, localized cervical cancer is effectively treated by surgical resection, while advanced cases are treated with radiation therapy along with platinum-based chemotherapy (Scholl et al., 2011).

Early studies showed that only 0.01-1% of cultured mouse myeloma cells formed colonies (Park et al., 1971), A number of later studies showed that a small proportion of tumor cells, designated as cancer stem cells, were capable of self-renewal and played a decisive role in tumor formation and growth (Lapidot et al., 1994; Reya et al., 2001). Tumor recurrence after conventional therapy has also been suggested to be due to the presence of these slow-cycling, cancer stem cells, which are capable of

initiating and sustaining neoplastic growth (Sagar et al., 2007; Ghaffari, 2011). The resistance of cancer stem cells to conventional chemotherapy and radiotherapy has been attributed to cellular mechanisms such as multidrug resistance, quiescence, enhanced DNA repair abilities and anti-apoptotic mechanisms (Saini and Shoemaker, 2010). Dysregulation of key signaling pathways such as the Wnt, Hedgehog, Notch and TGF-β/BMP pathways plays an important role in modulating tumorigenesis as well as stem cell function, suggesting that tumors may originate from transformed normal stem cells (Logan and Nusse, 2004). These studies suggest that identification and targeting of cancer stem cells are key aspects to optimizing cancer therapy.

Cancer stem cells have been isolated and cultured as structured spheroids in the presence of serum and growth factors (Saini and Shoemaker, 2010). However, a number of markers have been proposed to identify and isolate cancer stem cells including CD133, CD44, CD24, CD90, CD34, CD117, CD20, and aldehyde dehydrogenase (ALDH1) (Saini and Shoemaker, 2010; Friedman and Gillespie, 2011). ALDH1, a cytosolic enzyme, is responsible for oxidation of retinaldehydes to retinoids (Vasiliou et al., 2000). Although ALDH1 has been shown to be a cancer stem cell marker of breast cancer, head

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and neck squamous cell carcinoma, lung cancer, prostate cancer, pancreatic cancer, bladder cancer, and colorectal cancer (Schnier et al., 1999; Sophos and Vasiliou, 2003; Ma et al., 2008; Croker et al., 2009; Jiang et al., 2009; Ma et al., 2008; Su et al., 2010), its expression in cervical cancer has not been established.

The use of ALDH1 as a target molecule to select cancer stem cells has been facilitated by a combination of live cell ALDH1 activity detection and cell sorting techniques (Storms et al., 2005; Moreb et al., 2007, 2012). Fluorescent Aldefluor reagent (BAAA) is a non-toxic substrate that diffuses into living cells and is broken down to BAA by ALDH (Storms et al., 2005). The amount of intracellularly accumulated ALDH fluorescent reaction products is directly proportional to ALDH activity. Aldefluor reaction products are retained within cells with intact membranes, which are detected as ALDH-positive, while cells with increased permeability and dead cells are detected as ALDH-negative (Storms et al., 2005).

ALDH1 has been proposed as a diagnostic marker, a therapeutic target as well as a prognostic marker in a number of cancers (Heerma et al., 2011; Vogler et al., 2011; Wang et al., 2012). However, its functional role remains unclear. In this study, we investigated the expression of ALDH1 in cervical cancer cells and evaluated its clinical significance by exploring its role in tumorigenesis and metastasis.

Materials and Methods

Cell lines and animals

SiHa, HeLa and CaSki cervical carcinoma cell lines were obtained from Cell Resource Center, Shanghai Institute of Life Sciences, Chinese Academy of Sciences. The animals used in this study were 5 week old female NOD/SCID mice with an average body weight of 20 g and were purchased from Beijing HFK Bioscience Co., Ltd., China. All animal experimentation protocols were approved by the Animal Care and Use Committee.

Immunofluorescence

SiHa cell grew on coverglass were rinsed in PBS and then fixed for 30 mins in 4% formaldehyde at room temperature (RT, #4). The cells were later rinsed 3 times in PBS and then permeabilized by 0.1% Triton X-100 for 15 mins in RT. The fixed cells were incubated in primary antibodies overnight at 4 °C. After washing in PBS, the cells were then incubated with 2nd antibodies for 1 hours in RT. After wash, the coverglass were mounted and observed under confocal microscope.

Evaluation of ALDH1 expression using the Aldefluor method

Logarithmically growing Siha, HeLa and CaSki cells were harvested by trypsinization and evaluated for ALDH1 expression using the Aldefluor kit (Stem Cell Technology, USA), according to the manufacturer's instructions. Briefly, trypsinized cells were pelleted and resuspended in Aldefluor reagent at a density of 1×10^6/ml. The cells were incubated with Aldefluor substrate at 37 °C for 30 minutes and pelleted at 250xg for 5

mins. ALDH1 inhibitor, DEAB, was used to control for background fluorescence. Cell pellets were resuspended in Aldefluor detection buffer and ALDH1 expression was evaluated by flow cytometry (BD, USA).

Microsphere culture

Cells were seeded at a density of 1000 cells/ml in a 25 cm^3 culture flask containing 10 ml of complete DMEM-F12 medium (Gibco, USA) supplemented with B27 (Gibco, USA), 20 ng/mL EGF (BD Biosciences, USA), 5 µg/mL insulin (Sigma, USA) and 0.4% BSA (Sigma, USA). The flasks were incubated upright at 37 °C in the presence of 5% CO_2 incubator and horizontally shaken at least twice every day. Cell suspensions were centrifuged at 800 rpm for 5 min every 4 days and supernatant was discarded. The microspheres were resuspended in 10 ml fresh medium.

After 10-15 days of culture, the microsphere-containing medium was centrifuged at 800 rpm/min for 5 min and microspheres were passaged by digestion with 0.05% trypsin and 0.53 mM EDTA for 5-10 min followed by centrifugation at 1200 rpm/min for 5 min. Cells were resuspended in complete medium at a density of 1000 cells/ml.

Evaluation of ALDH1 expression on microsphere formation rates in SiHa cells

Aldefluor staining and flow cytometry were performed in order to sort the SiHa cells into ALDH1-positive and ALDH1-negative groups from the unsorted cells. Siha cell microspheres from the different groups were cultured for 14 days as described above and microspheres containing at least fifty cells or with a $> 50 \mu m$ diameter were counted and photographed under an inverted microscope. The microsphere formation rate (%) = number of microspheres/number of cells × 100%. Three replicates were used for each group, and the experiments were repeated 3 times.

MTT cell proliferation assay

SiHa cells were microsphere cultured for 14 days and 3 experimental groups were set up based on the Aldefluor staining and flow cytometry results; 1) unsorted SiHa cells, 2) SiHa-ALDH1-positive, and 3) SiHa-ALDH1-negative cells. Cell proliferation was measured using the MTT assay as follows. SiHa cells were plated at a density of 7000 cells/well in triplicate in 96 well plates. The MTT assay was performed on days 1, 3, 5 and 7 after seeding, by adding 20 μl of MTT working solution and incubating plates in the dark at 37 °C for 4h. The MTT-medium mixture was aspirated and DMSO (150 μl/well) was added and the plates shaken for 5 min to dissolve the blue-violet crystals. OD was measured at 492 nm.

Detection of the in vitro cell invasion capacity (metastasis) using a Transwell invasion chamber

SiHa cells were microsphere cultured for 14 days and 3 experimental groups were set up based on the Aldefluor staining and flow cytometry results; 1) unsorted SiHa cells, 2) SiHa-ALDH1-positive, and 3) SiHa-ALDH1-negative cells. The in vitro cell invasion capacity was measured. Briefly, the upper surface of the microporous polycarbonate membrane (pore size 8 µm) of the Transwell

invasion chamber (Costar Corp., USA) was coated with 20 μl of 0.5 g/L artificial Matrigel (BD Bioscience, USA) at 370C for 30 min. Serum-free high-glucose DMEM (Gibco, USA) was added to the chamber and 1 ml of high-glucose DMEM with 20% FBS in the basement. SiHa cells (1×10^5 cells/200 μl) were seeded in the chamber and incubated at 37 °C for 12 h. The chamber was removed, soaked and washed twice in PBS followed by 4% paraformaldehyde fixation for 15 min. The cells on the basement membrane were washed twice with PBS, gently wiped with a cotton swab and observed under an inverted microscope (5 fields were observed at 200 X magnification and averaged). The expresiment was repeated thrice with three replicates/sample.

Tumorigenicity experiments in NOD/SCID mice

SiHa cells were microsphere cultured for 14 days and 3 experimental groups were set up based on the Aldefluor staining and flow cytometry results; 1) unsorted SiHa cells, 2) SiHa-ALDH1-positive, and 3) SiHa-ALDH1-negative cells. Five-week-old female NOD/SCID mice were subcutaneously inoculated (n = 3 in each group) with different numbers of cells from each experimental group. The mice were sacrificed two weeks after tumor cell inoculation and tumor formation was evaluated.

Lentiviral packaging and cell transfection

293T cells were cultured to 40% confluence and transfected with the psPAX and pMD2.G lentiviral packaging systems. The total amount of transfected plasmid was 40 μg at a concentration ratio of 4:3:1 (lentiviral vector:psPAX:pMD2.G) (Cyagen, USA). The pSIN-EF2-IRES-GFP-puro lentiviral expression vector and auxiliary plasmid and the pshRNA-copGFP-puro siRNA expression vectors were obtained from System Bio, USA.

The ALDH1 gene-targeting oligonucleotide was designed by scanning the coding sequence of human ALDH1 cDNA using the Takara and Promega siRNA Target Designer. Based on the principles of siRNA target sequence design, the target sequence of the ALDH1 gene siRNA was eventually identified as follows:

Upstream: GATCTCCAGCAAGAAAT TTCCTGTCTTTATTCAAGAGATAAAGACAG GAAATTTC TTGCCTTTTTG and Downstream:AA TTCAAAAAAGCAAGAAATTTCCTGTCTTTATC TCTTGAATAAAGACAGGAAATTTCT TGCCGGA.

The supernatant (virus) was harvested after 24 h and stored at -800C. Virus solution was mixed with 2.5 ml high-glucose DMEM containing 10% FBS and polybrane was added to a final concentration of 4 μg/μL. SiHa cells were cultured to 40% confluence and transfected with virus solution. The infection step was repeated after 4 hours. Transfection efficiency was measured by fluorescence microscopy to determine GFP expression 2 days after the second infection. Real-time PCR was used to determine ALDH1 expression levels in the different groups of lentiviral transduced cells (Takara, Japan). The primers used for real-time PCR reactions were ALDH1 (F): GCACGCCAGACTTACCTGTC, ALDH1 (R): CCACTCACTGAATCATGCCA, GAPDH

(F): AAGGTGAAGGTCGGAGTCAA and GAPDH (R): AATGAAGGGGTCATTGATGG.

Statistical analyses

Normally distributed continuous variables were compared by one-way analysis of variance (ANOVA). When a significance between groups was apparent, multiple comparisons of means were performed using the Bonferroni procedure with type-I error adjustment. Data are presented as means ± standard deviation (SD). Repeated measurement with linear mixed model was carried out to determine the group effect in the change of MTT OD values among the groups. All statistical assessments were two-sided and evaluated at the 0.05 level of significant difference. Statistical analyses were performed using SPSS 15.0 statistics software (SPSS Inc, Chicago, IL).

Results

ALDH1 expression and localization in cervical cancer cell lines

We used indirect immunofluorescence assays to evaluate the expression and localization of ALDH1 in CaSki, SiHa and HeLa cells. Human hepatoma HepG2 cells, which are known to express high levels of ALDH1, were used as a positive control (Figure 1). ALDH1 was localized in the cytoplasm. We showed significant differences in ALDH1 expression in the different cell lines. HepG2 cells exhibited significantly higher levels of ALDH1 compared to CaSki, HeLa, and SiHa cervical cancer cells. Among the cervical cancer cell lines, ALDH1 expression was highest in CaSki cells and lowest in SiHa cells (Figure 1E).

Western blotting to determine ALDH1 expression levels in cervical cancer cell lines

We used immunoblotting to evaluate the expression of ALDH1 protein in CaSki, SiHa and HeLa cells. HepG2 cells, used as a positive control, exhibited the highest expression of ALDH1. CaSki cells expressed the highest levels of ALDH1, while SiHa cells expressed the lowest
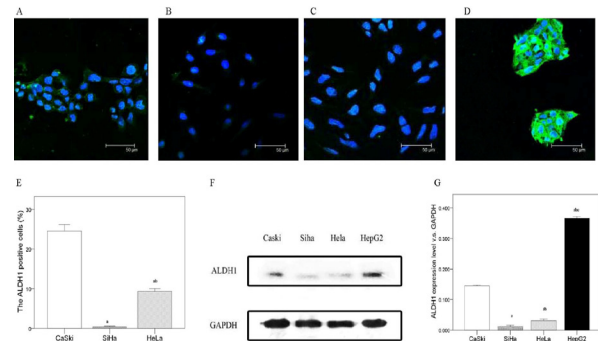

Figure 1. Detection of ALDH1 Expression in Cervical Cancer Cell Lines. Immunofluorescent detection of ALDH1 expression in (A) CaSki, (B) SiHa, (C) HeLa and (D) HepG2. (E)Quantitation of immunofluorescence data by counting the number of ALDH1-positive cells per field. (F) Western blot data to show ALDH1 expression in CaSki, SiHa, HeLa and HepG2 cells. GAPDH was used as the normalizing control. (G) Quantitation of immunoblot data
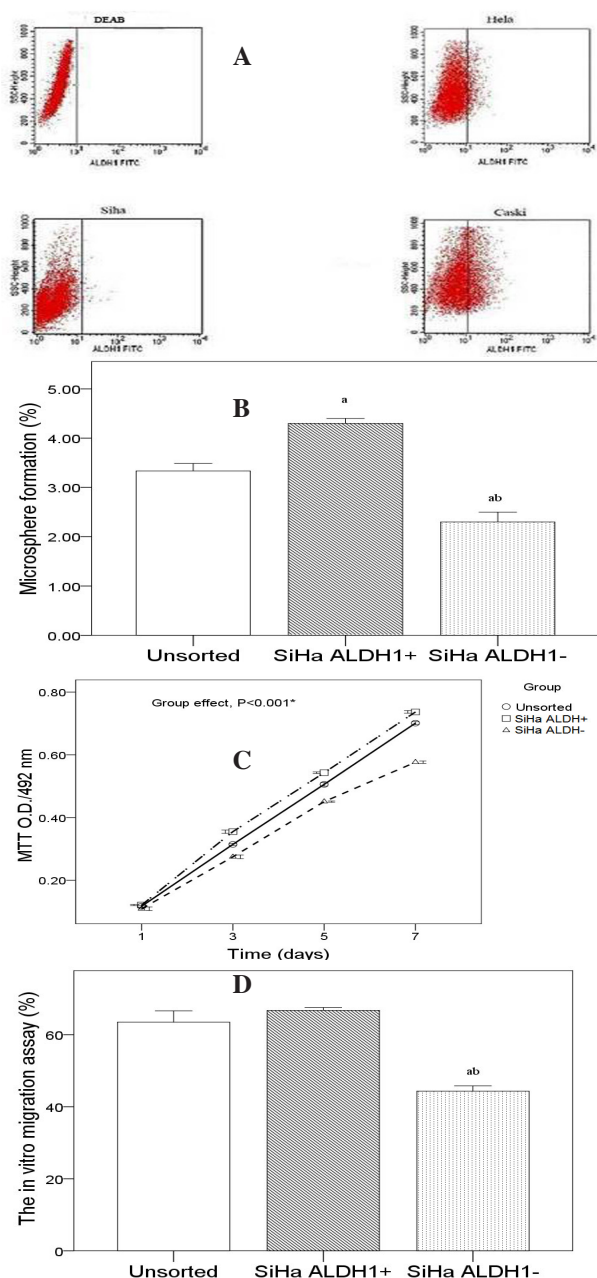
Figure 2. Charaterization of ALDH1 Positive Cervical Cancer Cells. (A) Aldefluor staining and cell sorting results. Top left panel: cells with ALDH1 inhibitor. Top right panel: HeLa cells. Lower Left panel: SiHa cells. Lower right panel: CaSki cells. (B) Microsphere formation rates of different cell lines. (C) MTT assay to evaluate cell proliferation of different cell lines. (D) Evaluation of cell migration rates of different cell lines

levels of ALDH1 (Figure 1F).

Quantitation of the western blot data showed a significantly higher expression of ALDH1 protein in HepG2 cells, followed by CaSki cells (Figure 1G). ALDH1 expression levels were similar in SiHa cells and HeLa cells (Figure 1G).

Aldefluor staining to determine ALDH1 expression in the cervical cancer cell lines

We used Aldefluor staining followed by flow cytometry to determine the percentage of ALDH1-positive cells in cultured HeLa, CaSki, and SiHa cells (Figure 2A).. Our data analysis showed significant differences in the proportion of ALDH1-positive cells in the three cell
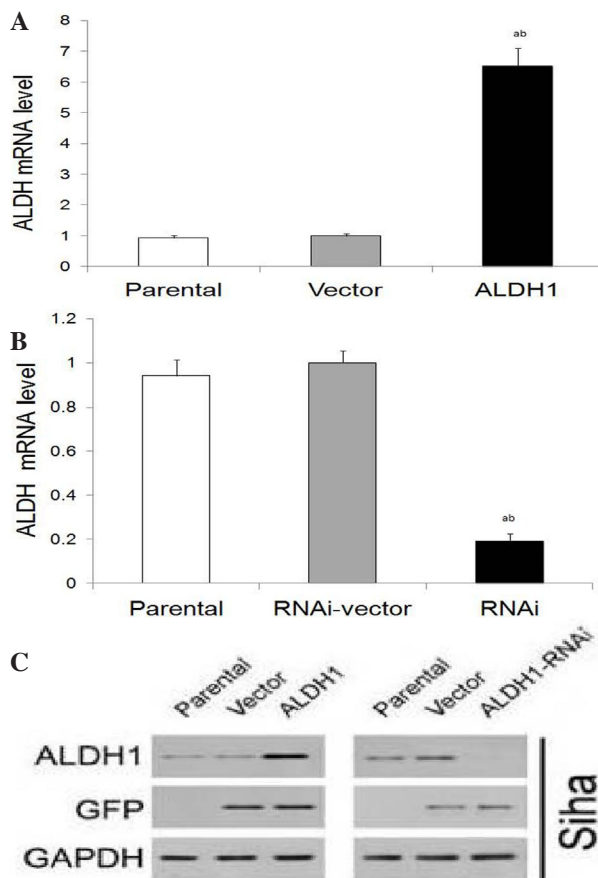
Figure 3. Altering ALDH1 Gene Expression Using Lentiviral Vectors. (A) Detection of ALDH1 mRNA levels in SiHa cells transduced with ALDH1-overexpressing lentiviral vector. (B) Detection of ALDH1 mRNA levels in SiHa cells transduced with ALDH1-knockdown lentiviral vector. (C) Detection of ALDH1 protein by western blot in SiHa cells transduced with ALDH1-overexpressing or knockdown lentiviral vectors

lines. CaSki cells had the highest percentage of ALDH1-positive cells, while SiHa cells had the lowest percentage of ALDH1-positive cells (CaSki 24.55 ± 1.58%, HeLa 9.33 ± 0.68%, and SiHa 0.41 ± 0.2%)

Effect of ALDH1 expression on microsphere formation in Siha cells

SiHa cell microspheres were cultured in suspension for 7-14 days and subjected to Aldefluor staining and flow cytometry sorting. After sorting, the SiHa cells were divided into three different groups 1) unsorted SiHa cells 2) SiHa-ALDH1-positive, and 3) SiHa-ALDH1-negative cells.

Cell microsphere formation rate

Unsorted SiHa cells, SiHa-ALDH1-positive cells, and SiHa-ALDH1-negative cells, were seeded in ultra-low adhesion 24-well plates at a density of 1000 cells/well and cultured for 14 days in triplicate. The microspheres were then counted, and photographed under the microscope. We found that SiHa-ALDH1-positive cells had a significantly higher rate of microsphere formation on Day 14, compared to the unsorted and ALDH1-negative cells. The microsphere-forming rate of SiHa-ALDH1-positive cells was significantly higher than unsorted SiHa cells
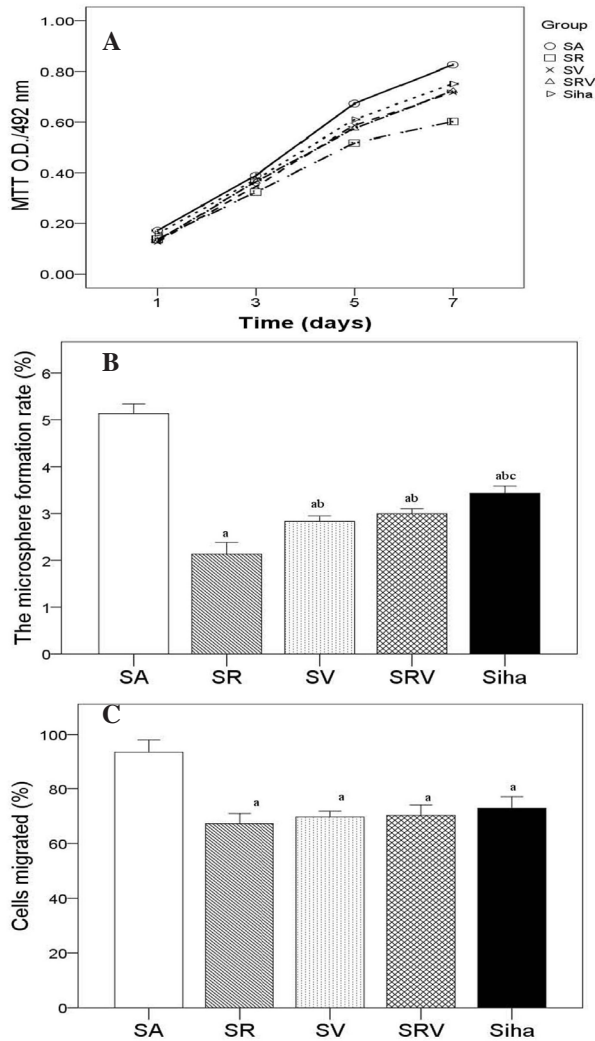
Figure 4. The Role of ALDH1 in the Tumorigenecity of SiHa Cells. (A) MTT assay to evaluate cell proliferation in SiHa cells transduced with ALDH1-overexpressing or knockdown lentiviral vectors. (B) Evaluation of microsphere formation rate of SiHa cells transduced with different lentiviral vectors. (C) Evaluation of migration rate of SiHa cells transduced with different lentiviral vectors

(4.30% vs. 3.33%) (P<0.01). The microsphere-forming rate of Siha-ALDH1-positive cells was almost twice that of Siha-ALDH1-negative cells (4.30% vs. 2.3%; P<0.01) (Figure 2B).

Effect of ALDH1 expression on cell proliferation and migration

We used the MTT and migration assays to compare proliferation and metastasis rates in SiHa-ALDH1-positive cells and SiHa-ALDH1-negative cells. We showed that unsorted and SiHa-ALDH1-positive cells had significantly higher proliferation and migration rates compared to SiHa-ALDH1-negative cells (Figures 2C, D).

Effect of ALDH1 expression on tumorigenesis

We evaluated the effect of ALDH1 expression on the tumor formation ability in nude mice. Subcutaneous injection of nude mice with SiHa-ALDH1-positive cells resulted in a higher number of tumors compared to mice injected with unsorted or SiHa-ALDH1-negative cells

Table 1. The Tumor Formation of Three Groups

Group	The number of NOD/SCID mice with tumor (n=3)			
	10^5	10^4	10^3	10^2
SiHa-ALDH+	3	2	1	0
SiHa-ALDH-	0	0	0	0
Unsorted	1	0	0	0

Table 2. The Tumor Formation of Three Groups after Transfection

Group	The number of NOD/SCID mice with tumor (n=3)			
	10^5	10^4	10^3	10^2
SA	3	3	1	0
SR	0	0	0	0
SV	1	0	0	0
SRV	1	0	0	0
SiHa	1	0	0	0

(Table 1).

Effect of ALDH1 overexpression and knockdown on stem cell properties in cervical cancer cells

We used lentiviral transduction to overexpress or knockdown ALDH1 expression in SiHa cells in order to investigate the role of ALDH1 expression on the stem cell properties of SiHa cells. We showed that, 97% of transduced SiHa cells expressed GFP, a marker of viral infection. We transduced SiHa cells with 1) lentiviral empty vector (SV) 2) lentiviral ALDH1-overexpression vector (SA), 3) lentiviral shRNA empty vector (SRV), 4) lentiviral shRNA ALDH1 vector (SR) or 5) no virus (SiHa). Using quantitative RT-PCR, we showed a six fold increase in ALDH1 mRNA levels in SA-transduced SiHa cells at 48 hours post virus transduction (Figure 3A), while the SR-transduced SiHa cells had an 80% reduction in ALDH1 mRNA levels (Figure 3B) compared to uninfected cells. Our western blotting data were consistent with the RT-PCR data and showed a significant increase in the levels of ALDH1 protein in SA-transduced SiHa cells and a significant decrease in SR-transduced SiHa cells compared to control cells (Figure 3C).

We evaluated cell proliferation, cell migration and microsphere formation properties in lentivirus-transduced SiHa cells. Our data showed a significant increase in cell proliferation, microsphere formation and cell migration in SA-transduced SiHa cells compared to control cells transduced with empty vector (Figure 4). However, although knockdown of ALDH1 using the SR vector resulted in a significant decrease in the SiHa cell proliferation and microsphere formation, there was no significant decrease in migration in the SR-transduced SiHa cells compared to empty vector-transduced cells (Figure 4).

We evaluated the ability of the lentiviral-transduced cells to form tumors in nude mice subcutaneously injected with the different groups of cells. We showed that SA-transduced SiHa cells were more potent at inducing tumor formation in nude mice compared to the empty vector control. However, tumor forming ability was abolished in mice injected with SR-transduced cells where ALDH1 expression was knocked down (Table 2).

Discussion

In this study, we evaluated ALDH1 expression in cervical cancer cells and showed that ALDH1-positive cells exhibited the properties of cancer stem cells. We investigated the role of ALDH1 in tumorigenesis and metastasis of three different cervical carcinoma cell lines. We showed that CaSki cells, which are invasive metastatic cervical cancer cells with the highest degree of malignancy, had the highest expression of ALDH1, followed by HeLa human cervical adenocarcinoma cells. SiHa (human cervical carcinoma) cells, which were the least malignant, were < 1% ALDH1-positive. This is very close to the percentage of cancer stem cells in a tumor. We also showed a significant increase in the tumorigenicity and migration of SiHa cells overexpressing ALDH1, compared to control SiHa cells. Knockdown of ALDH1 in SiHa-ALDH1-positive cells abolished the ability of these cells to form tumors in nude mice. ALDH1 expression has been previously used as a prognostic marker, a diagnostic marker and a marker or drug resistance in a number of tumors (Schnier et al., 1999; Vogler et al., 2011; Heerma van Voss et al., 2011; Wang et al., 2011; Moreb et al., 2012; Wang et al., 2012). However, to the best of our knowledge, we are the first to demonstrate the role of ALDH1 in tumorigenesis and metastasis in cervical cancer.

ALDH1 is a cytosolic enzyme which catalyzes the irreversible oxidation of retinol to retinoic acid (Yoshida et al., 1992) and is thought to play a role in cellular differentiation via the retinoid pathway (Ginestier et al., 2009). ALDH1 has been shown to regulate hematopoiesis by promoting myeloid differentiation (Rice et al., 2008). ALDH1 also played a role in neural stem cell differentiation (Corti et al., 2006).

In this study, we selected and enriched the ALDH1$^+$ population of SiHa cervical carcinoma cells by Aldefluor staining followed by flow cytometry. We showed that SiHa-ALDH1-positive cells had a significantly higher rate of microsphere formation compared to SiHa-ALDH1-negative cells, indicating the cancer stem cell properties of the ALDH1-enriched population. We also showed that the SiHa-ALDH1-positive cells had significantly higher rates of proliferation and migration, compared to the SiHa-ALDH1-negative cells. Our data were consistent with earlier studies demonstrating a positive correlation between ALDH1 expression and cellular proliferation rates (Jiang et al., 2009; Morimoto et al., 2009; Kahlert et al., 2011). Since normal stem cells are largely quiescent, it will be interesting to investigate the mechanism underlying ALDH1-mediated increase in the proliferation of cancer stem cells by exploring its effect on key cell cycle events.

Our data also agreed with previous research showing that ALDH1 expression was upregulated in diffuse-type lymph node metastasis compared to the primary tumor in gastric cancer, esophageal cancer and breast cancer (Charafe-Jauffret et al., 2010; Wakamatsu et al., 2012; Wang et al., 2011). We evaluated the role of ALDH1 on metastasis by comparing the migration of SiHa-ALDH1-positive with that of SiHa-ALDH1-negative cells. SiHa-ALDH1-positive cells had significantly higher migration rates compared to SiHa-ALDH1-negative cells,

suggesting that ALDH1 may play a role on metastasis of cervical cancer. Our future goals include comparisons of ALDH1 expression in metastatic cervical carcinoma patients with non-metastatic patients.

Only a small number of solid tumor cells are capable of forming colonies in in-vitro clonogenic assays, suggesting that a rare subset of tumor cells have the ability to form new tumors (Reya et al., 2001). Isolation and characterization of such cells are key to understanding molecular mechanisms underlying their tumorigenic capacity. ALDH1-positive cells were isolated from four different breast cancer cell lines and were shown to be tumorigenic when inoculated into nude mice (Croker et al., 2009). We evaluated the role of ALDH1 on tumorigenesis in mice inoculated with 1) SiHa-ALDH-positive and SiHa-ALDH1-negative cells or 2) SiHa cells with lentiviral-mediated overexpression or knockdown of ALDH1. Tumor formation was seen with as few as 1000 SiHa-ALDH1-positive cells or ALDH1-overexpressing SiHa cells. However, this tumor forming ability was abolished when ALDH1 expression was knocked down in SiHa cells, suggesting ALDH1 played an important role in tumorigenesis and that ALDH1-positive SiHa cells constituted the subset of SiHa cancer stem cells. We compared microsphere formation, cell proliferation and migration in SiHa cells overexpressing ALDH1 with SiHa cell in which ALDH1 was knocked down. We showed that ALDH1 overexpression resulted in a significant increase in microsphere formation, cell proliferation as well as migration.

An important limitation of our study lies in the fact that we did not use a different stem cell marker such as CD133 or CD44, in order to validate our data. We plan to address this question in our future studies. Although a previous study demonstrated ALDH1 expression in cervical carcinoma cells (Yao et al., 2011), suggesting the presence of cancer stem cells, we are the first to show that ALDH1-positive cervical cancer cells were significantly more tumorigenic and invasive compared to ALDH1-negative cells. Our future studies aim to dissect the mechanisms underlying ALDH1-mediated increase in proliferation, invasion and tumorigenesis in vitro and in cervical carcinoma tissue from metastatic and non-metastatic cervical carcinoma patients. Our present study expands our understanding of the role of ALDH1 in cancer stem cells.

Acknowledgements

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