

# MCT1 gene silencing enhances the immune effect of dendritic cells on cervical cancer cells

Xiaoxin Sui<sup>1,A–F</sup>, Xiaowei Xi<sup>2,B,C</sup>

<sup>1</sup> Department of Obstetrics and Gynecology, Shanghai Changzheng Hospital, Naval Medical University, Shanghai, China

<sup>2</sup> Department of Obstetrics and Gynecology, General Hospital, Shanghai Jiao Tong University School of Medicine, China

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;

D – writing the article; E – critical revision of the article; F – final approval of the article

Advances in Clinical and Experimental Medicine, ISSN 1899–5276 (print), ISSN 2451–2680 (online)

Adv Clin Exp Med. 2024;33(7):739–749

## Address for correspondence

Xiaoxin Sui

E-mail: suixiaoxinsjtu@126.com

## Funding sources

The study was funded by the National Natural Science Foundation of China (grant No. 81772767) and the Science and Technology Commission of Shanghai Municipality (grant No. 15411952300).

## Conflict of interest

None declared

Received on August 29, 2022

Reviewed on March 1, 2023

Accepted on August 21, 2023

Published online on January 5, 2024

## Abstract

**Background.** Dendritic cells (DCs) are a key class of immune cells that migrate to the draining lymph nodes and present processed antigenic peptides to lymphocytes after being activated by external stimuli, thereby establishing adaptive immunity. Moreover, DCs play an important role in tumor immunity.

**Objectives.** The aim of the study was to investigate whether *MCT1* gene silencing in DCs affects their ability to mount an immune response against cervical cancer cells.

**Materials and methods.** We silenced the expression of *MCT1* in DCs from mouse bone marrow (BM) by infection with adenovirus. The surface antigen profile of DCs was analyzed by flow cytometry and cytokine secretion was evaluated using enzyme-linked immunosorbent assay (ELISA) following sodium lactate (sLA) exposure and lipopolysaccharide (LPS) stimulation. Then, various groups of DC-induced cytotoxic T lymphocytes (CTLs) were prepared and their cytotoxicity against U14 was tested.

**Results.** Without sLA exposure, silencing *MCT1* did not affect the expression of CD1a, CD80, CD83, CD86, and major histocompatibility complex class II (MHCII) in DCs after LPS challenge. Similar results were found for interleukin (IL)-6, IL-12 p70 and tumor necrosis factor alpha (TNF- $\alpha$ ). After sLA exposure, silencing *MCT1* significantly decreased the expression of CD1a, CD80, CD83, CD86, and MHCII in DCs after the LPS challenge, as well as the secretion of IL-6, IL-12 p70 and TNF- $\alpha$ . In addition, sLA exposure significantly reduced the toxicity and inhibited the proliferation of DC-induced CTLs compared to U14 cells in vitro and in vivo. However, silencing *MCT1* significantly attenuated the changes caused by sLA exposure. At the same time, in the absence of sLA, silencing *MCT1* did not affect the toxicity nor inhibit the proliferation of DC-induced CTLs on U14 cells.

**Conclusions.** Lactate exposure reduces the immune effect of DCs on cervical cancer cells, but *MCT1* gene silencing attenuates these alterations.

**Key words:** dendritic cells, cervical cancer, immunity, *MCT1*

## Cite as

Sui X, Xi X. *MCT1* gene silencing enhances the immune effect of dendritic cells on cervical cancer cells. *Adv Clin Exp Med*. 2024;33(7):739–749. doi:10.17219/acem/171446

## DOI

10.17219/acem/171446

## Copyright

Copyright by Author(s)

This is an article distributed under the terms of the Creative Commons Attribution 3.0 Unported (CC BY 3.0) (<https://creativecommons.org/licenses/by/3.0/>)

## Background

Dendritic cells (DCs) represent a critical subset of immune cells that initiate adaptive immunity by migrating to the draining lymph nodes. These cells are activated by external stimuli and present processed antigenic peptides to lymphocytes.<sup>1,2</sup> Moreover, DCs play an essential role in tumor immunity by ingesting tumor antigens and maturing to express major histocompatibility complex (MHC) class I and II molecules, costimulatory factors, and adhesion factors.<sup>3,4</sup> In addition, DCs combined with T cells induce the killer cells to produce large quantities of interferon (IFN)- $\gamma$ , perforin and granzyme by secreting high concentrations of interleukin (IL)-12, thereby enhancing the lysis effect of target cells on cancer cells.<sup>3,4</sup> Simultaneously, DCs can induce the killing effect of CD8+ T cells, facilitating the clearance of antigen-specific tumor cells. Thus, DCs initiate the body's anti-tumor immunity and are the bridge between T cells and tumor cells.<sup>5,6</sup> Any alteration to the normal functioning of DCs can directly impact the body's anti-tumor immunity.

Tumor immunotherapy, especially chimeric antigen receptor (CAR)-T cell immunotherapy, has developed rapidly in recent years.<sup>7</sup> Despite significant clinical outcomes of immunotherapies in liquid tumors, their effectiveness in solid tumors is comparatively limited. The microenvironment of solid tumors has been identified as a key factor contributing to the significant difference in clinical responses to cell-based immunotherapy between these two forms of cancers.<sup>7,8</sup> The Warburg Effect is the most notable feature distinguishing tumor cells from normal cells, in which tumor cells preferentially choose to supply energy through glycolysis under anaerobic conditions, and lactate is the main by-product of glycolysis energy supply for tumor cells.<sup>9,10</sup> The Warburg Effect may provide tumor cells with an escape mechanism against the immune system, as high lactate concentrations have been reported to alter the phenotype of immune cells.<sup>11–13</sup> A recent study stated that lactate exposure attenuated DC maturation by downregulating CD80 and MHCII expression following lipopolysaccharide (LPS) stimulation.<sup>13</sup> Therefore, lactate is a barrier affecting the anti-tumor immunity of DCs.

Monocarboxylate transporters (MCTs) of the SLC16 solute carrier family are key proteins for intracellular and extracellular lactate exchange.<sup>14–16</sup> Under physiological conditions, MCTs prevent lactate accumulation within cells by eliminating its excess produced due to increased glycolytic activity. This process has potential implications for developing cancer therapeutics targeting lactate metabolism.<sup>16,17</sup> Dendritic cells express several MCTs, including MCT1, MCT2 and MCT4. The MCT1 and MCT2 are responsible for transporting extracellular lactate into the cell, while MCT4 is responsible for transporting intracellular lactate outside the cell.

Additionally, while MCT1 and MCT4 are regulated by lactate, MCT2 is not involved in lactate management.<sup>13,15,18</sup>

Therefore, we hypothesized that the expression level of MCT1 in DCs would have an impact on their phenotype under high lactate conditions, thereby affecting their anti-tumor immunity. In this study, we used adenovirus to silence MCT1 of mouse bone marrow (BM)-derived DCs to investigate their phenotypic changes and toxicity to cervical cancer cells in standard or high lactate environments.

## Objectives

This study aimed to examine the impact of DC-mediated immunity on cervical cancer cells following *MCT1* gene silencing.

## Materials and methods

### Ethics statement

This study was carried out following the National Institutes of Health Guide for the Care and Use of Laboratory Animals.<sup>19</sup> The protocol was approved by the Animal Ethics Committee of Shanghai Changzheng Hospital, Naval Medical University, China (approval No. 2017KY068).

### Cells and reagents

The U14 cells were purchased from American Type Culture Collection (Manassas, USA) and cultured in RPMI-1640 medium (A1049101; Gibco, Waltham, USA) with the addition of 10% fetal bovine serum (FBS) (10099141; Gibco) at 37°C with 5% CO<sub>2</sub>. Mouse SLC16A1 (*MCT1*) shRNA silencing adenovirus (Ad-sh*MCT1*) and its matched control shRNA adenovirus were purchased from Vector Biolabs (shADV-272089; Vector Biolabs, Malvern, USA). The anti-*MCT1* antibody was purchased from Abcam (ab156080; Cambridge, UK). Other used antibodies included FITC-conjugated CD1a antibody (ab27992; Abcam), CD80 antibody (ab18279; Abcam), CD83 antibody (MHCD8301; Thermo Fisher Scientific, Waltham, USA), CD86 antibody (MHCD8601; Thermo Fisher Scientific), and MHCII antibody (11-9956-42; eBioscience, San Diego, USA). The Cell Counting Kit-8 (CCK-8) (C0037), mouse IL-6 enzyme-linked immunosorbent assay (ELISA) kit (PI326) and mouse tumor necrosis factor alpha (TNF- $\alpha$ ) ELISA kit (PT512) were purchased from Beyotime Biotechnology (Shanghai, China). Finally, the mouse IL-12 p70 ELISA kit (EK0500) was purchased from Signalway Antibody (Greenbelt, USA).

### Adenovirus infection of BM-derived DCs

As previously described,<sup>20</sup> we prepared DCs from mouse BM. In brief, the mouse tibia was rinsed with phosphate-buffered saline (PBS), red blood cells were fully lysed, and

cells were collected by centrifugation. The cells from BM were cultured in RPMI-1640 medium supplemented with 10% FBS, 20 ng/mL rmGM-CSF (PMC2016; Thermo Fisher Scientific) and 20 ng/mL rmIL-4 (RMIL4I; Thermo Fisher Scientific) at 37°C with 5% CO<sub>2</sub> for 1 week. Subsequently, Ad-shCtrl or Ad-shMCT1 adenoviruses (MOI = 10:1) were added to BM-derived DCs for 12 h, cultured at 37°C with 5% CO<sub>2</sub>. Then, the medium was replaced with a fresh one to continue culturing.

## Immunoblot analysis

After 24 h of Ad-shCtrl or Ad-shMCT1 infection, DCs were harvested, and an appropriate amount of cell lysing solution was utilized to extract total cellular protein. Approximately 40 µg of cellular protein was analyzed using 10% sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE), followed by the transfer of protein molecules to a polyvinylidene difluoride (PVDF) membrane. Subsequently, the membrane was blocked with 5% non-fat milk powder at room temperature for 1 h and then incubated overnight at 4°C with the MCT1 antibody. After being washed 3 times with tris-buffered saline (TBS)+0.1% Tween-20 (TBST) buffer at room temperature, secondary antibodies were added to the membrane and it was incubated for 1 h at room temperature. Protein bands were developed using an ECL Chemiluminescence Kit (P0018FS; Beyotime), and the protein band grey value was analyzed using ImageJ v. 1.8.0 (<https://imagej.nih.gov/ij/index.html>).

## Dendritic cell phenotype analysis and cytokine assay

After 24 h of Ad-shCtrl or Ad-shMCT1 infection, 50 mM of sodium lactate (sLA) was added to the culture medium for 48 h (an equal amount of PBS was used as control) and then changed to a fresh medium supplemented with 1 µg/mL LPS (L2880; Sigma-Aldrich, St. Louis, USA), followed by culturing for another 24 h. Then, we collected DCs and analyzed their phenotype using a flow cytometer. The cell culture medium was collected and investigated for cytokine content (IL-6, IL-12 p70 and TNF-α), in accordance with the manufacturer's instructions.

## Cytotoxicity assay

Dendritic cells were co-cultured with mouse splenic T cells for 1 week at a ratio of 1:10 after adenovirus infection, sLA exposure (PBS as control) and LPS challenge. Then, we collected the T cells after 7 days, defining them as cytotoxic T lymphocytes (CTLs). Using a CCK-8 kit, the in vitro cytotoxicity of DC-induced CTLs was assayed by culturing the CTLs with target U14 cells for 24 h at effector:target (E:T) ratios of 90:1, 30:1 and 10:1.

## Cell clone formation test

The DC-induced CTLs and U14 cells were mixed and cultured (1:10), and after 24 h of culture, they were digested with trypsin. The cells were resuspended as a single-cell suspension, and 1000 cells were seeded into a 6-centimeter culture dish and incubated in a culture medium at 37°C with 5% CO<sub>2</sub> for 14 days. Then, the cells were stained with crystal violet and the number of clones was counted.

## Nude mouse U14 cell xenograft

After a week of adaptive feeding, 42 nude mice (6–8 weeks old, 18–22 g) were randomly divided into 6 groups. The U14 cells in the ratio of 2×10<sup>6</sup> cells/100 µL were injected subcutaneously into the back of the mice. After 10 days, 2×10<sup>6</sup> cells/200 µL of DC-induced CTLs were injected into U14 xenografts for treatment once a week for a period of 30 days. Subsequently, mice were euthanized, and the U14 xenografts were isolated and compared for tumor tissue weight.

## Statistical analyses

The SPSS v. 20.0 (IBM Corp., Armonk, USA) and GraphPad Prism (v. 5.0; GraphPad Software, Boston, USA) were used to analyze data. The measurement data were expressed as mean ± standard deviation. We used the Shapiro–Wilk test to assess whether the measured data conform to the normal distribution in SPSS. The p-value was calculated using analysis of variance (ANOVA) followed by Tukey's multiple comparison test between multiple groups in GraphPad Prism. Student's t-test was used to compare the differences between 2 groups. A value of p < 0.05 was considered statistically significant.

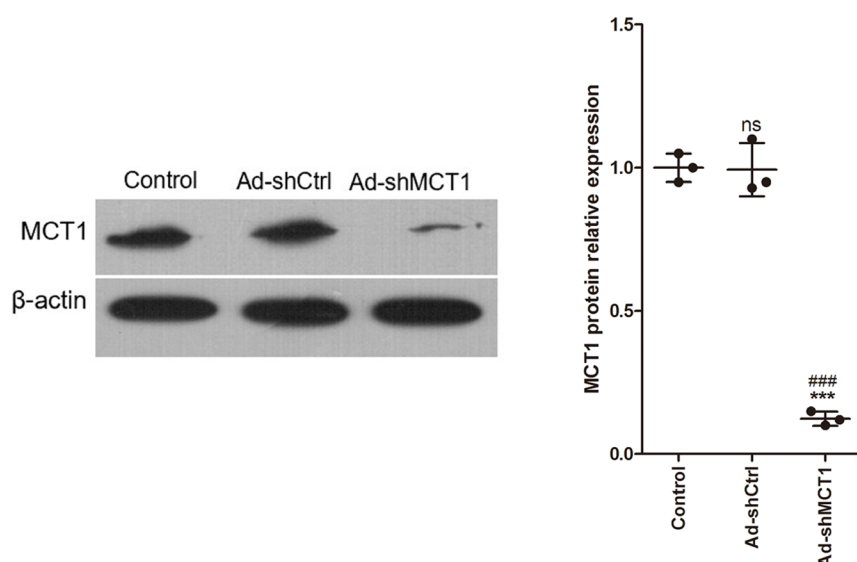
## Results

### Analysis of silencing MCT1 in DCs

Immunoblotting was used to detect the expression of MCT1 protein in DCs in order to analyze the *MCT1* gene silencing effect of Ad-shMCT1 adenovirus. Results showed that Ad-shMCT1 adenovirus significantly decreased the expression of MCT1 protein in DCs compared to the control group (p < 0.05, honestly significant difference (HSD) test following ANOVA, Fig. 1 and Table 1).

### MCT1 silencing alters the phenotype of mature DCs following sLA exposure

In the absence of sLA, MCT1 silencing did not affect the percentage of DCs expressing CD1a, CD80, CD83, CD86, and MHCII. Upon sLA exposure and MCT1



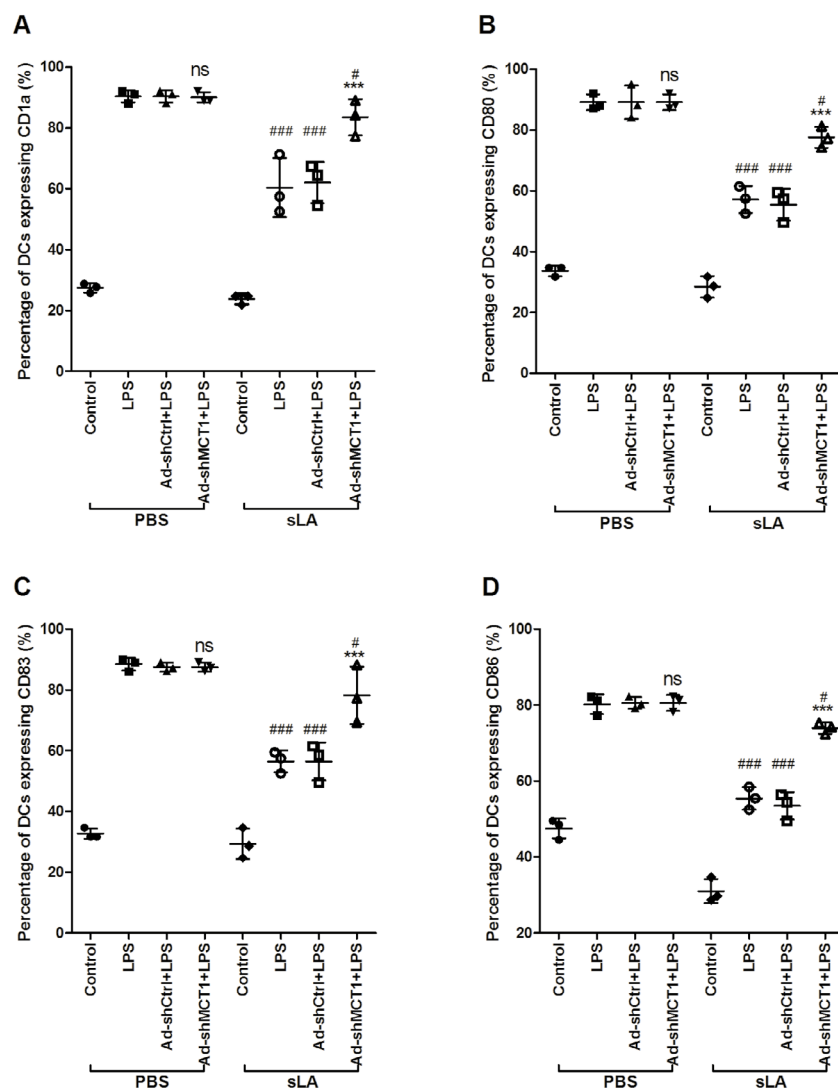
**Fig. 1.** Analysis of MCT1 inhibition in dendritic cells (DCs). We used immunoblotting to analyze the inhibition efficacy of mouse SLC16A1 (MCT1) shRNA silencing adenovirus (Ad-shMCT1) on the expression of MCT1 in DCs, and ImageJ software was used to analyze the relative MCT1 expression. The column height represents the average value, and the error bar represents the standard deviation. The p-value was calculated by Tukey's honestly significant difference (HSD) test following the analysis of variance (ANOVA). We performed 3 independent replicates for MCT1 protein detection in each group

<sup>ns</sup>  $p > 0.05$ , ###  $p < 0.001$  compared to the control group; \*\*\*  $p < 0.001$  compared to the Ad-shCtrl group.

**Table 1.** Statistics for Fig. 1

	Variable	Minimal	Q1	Median	Q3	Maximal	Mean	SD
MCT1	control (n = 3)	0.95	0.95	1.00	1.05	1.05	1.00	0.05
	Ad-shCtrl (n = 3)	0.93	0.93	0.95	1.10	1.10	0.99	0.09
	Ad-shMCT1 (n = 3)	0.10	0.10	0.12	0.15	0.15	0.12	0.025

SD – standard deviation; Q1 – 1<sup>st</sup> quartile; Q3 – 3<sup>rd</sup> quartile; Ad-shMCT1 – mouse SLC16A1 (MCT1) shRNA silencing adenovirus.



**Fig. 2.** Percentage values of dendritic cells (DCs) expressing CD1a, CD80, CD83, CD86, and major histocompatibility complex class II (MHCII). Dendritic cells were first infected with Ad-shCtrl and mouse SLC16A1 (MCT1) shRNA silencing (Ad-shMCT1) adenoviruses for 24 h, then co-cultured with 50 mM of sodium lactate (sLA) or phosphate-buffered saline (PBS) for 48 h, and finally stimulated with 1  $\mu$ g/mL lipopolysaccharide (LPS) for 24 h. Flow cytometry was used to detect the expression of CD1a (A), CD80 (B), CD83 (C), CD86 (D), and MHCII (E) in DCs. The column height represents the average value, and the error bar represents the standard deviation. The p-value was calculated by Tukey's honestly significant difference (HSD) test following the analysis of variance (ANOVA) between multiple groups or by Student's t-test (PBS compared to sLA treatment)

<sup>ns</sup>  $p > 0.05$ , \*\*\*  $p < 0.001$  compared to the Ad-shCtrl+LPS group; #  $p < 0.05$ , ##  $p < 0.01$  and ###  $p < 0.001$  compared to the same treatment in the PBS group.

silencing, the percentage of DCs significantly increased ( $p < 0.05$ , HSD test following ANOVA, Fig. 2 and Table 2). However, after sLA exposure and LPS stimulation, the rate of DCs significantly decreased, without a concomitant change in DC viability (Supplementary Fig. 1, Supplementary Table 1).

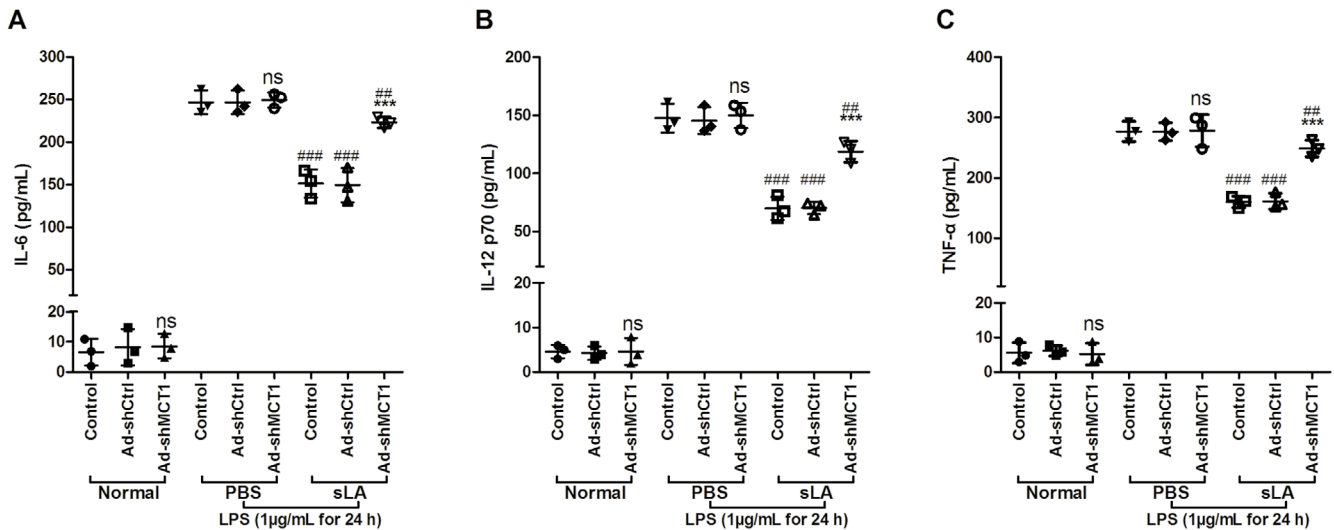
## MCT1 silencing alters the secretion of cytokine from DCs after sLA exposure

The MCT1 silencing significantly increased the DC levels of IL-6, IL-12 p70 and TNF- $\alpha$  after sLA exposure ( $p < 0.05$ , HSD test following ANOVA, Fig. 3 and Table 3).

Table 2. Statistics for Fig. 2 (n = 3)

Variable		Minimal	Q1	Median	Q3	Maximal	Mean	SD
PBS-CD1a	control	25.74	25.74	27.72	28.71	28.71	27.39	1.51
	LPS	88.12	88.12	91.09	92.08	92.08	90.43	2.06
	Ad-shCtrl+LPS	88.12	88.12	91.09	92.08	92.08	90.43	2.06
	Ad-shMCT1+LPS	89.11	89.11	89.11	92.08	92.08	90.1	1.72
sLA-CD1a	control	21.78	21.78	24.75	24.75	24.75	23.76	1.72
	LPS	52.48	52.48	57.43	71.29	71.29	60.40	9.75
	Ad-shCtrl+LPS	54.46	54.46	64.36	67.33	67.33	62.05	6.74
	Ad-shMCT1+LPS	77.23	77.23	84.16	89.11	89.11	83.50	5.97
PBS-CD80	control	31.68	31.68	34.65	34.65	34.65	33.66	1.72
	LPS	87.13	87.13	88.12	92.08	92.08	89.11	2.62
	Ad-shCtrl+LPS	84.16	84.16	88.12	95.05	95.05	89.11	5.51
	Ad-shMCT1+LPS	87.13	87.13	88.12	92.08	92.08	89.11	2.62
sLA-CD80	control	24.75	24.75	28.71	31.68	31.68	28.38	3.48
	LPS	52.48	52.48	57.43	61.39	61.39	57.1	4.46
	Ad-shCtrl+LPS	49.50	49.5	57.43	59.41	59.41	55.45	5.24
	Ad-shMCT1+LPS	74.26	74.26	77.23	81.19	81.19	77.56	3.48
PBS-CD83	control	31.68	31.68	31.68	34.65	34.65	32.67	1.72
	LPS	86.14	86.14	89.11	90.10	90.10	88.45	2.06
	Ad-shCtrl+LPS	86.14	86.14	87.13	89.11	89.11	87.46	1.51
	Ad-shMCT1+LPS	86.14	86.14	87.13	89.11	89.11	87.46	1.51
sLA-CD83	control	24.75	24.75	28.71	34.65	34.65	29.37	4.98
	LPS	52.48	52.48	57.43	59.41	59.41	56.44	3.57
	Ad-shCtrl+LPS	49.50	49.50	58.42	61.39	61.39	56.44	6.19
	Ad-shMCT1+LPS	69.31	69.31	77.23	88.12	88.12	78.22	9.44
PBS-CD86	control	44.55	44.55	48.51	49.50	49.50	47.52	2.62
	LPS	77.23	77.23	81.19	82.18	82.18	80.20	2.62
	Ad-shCtrl+LPS	79.21	79.21	80.20	82.18	82.18	80.53	1.51
	Ad-shMCT1+LPS	78.22	78.22	81.19	82.18	82.18	80.53	2.06
sLA-CD86	control	28.71	28.71	29.70	34.65	34.65	31.02	3.18
	LPS	52.48	52.48	55.45	58.42	58.42	55.45	2.97
	Ad-shCtrl+LPS	49.50	49.50	54.46	56.44	56.44	53.47	3.58
	Ad-shMCT1+LPS	72.28	72.28	74.26	75.25	75.25	73.93	1.51
PBS-MHCII	control	49.50	49.50	54.46	57.43	57.43	53.80	4.01
	LPS	93.07	93.07	95.05	98.02	98.02	95.38	2.49
	Ad-shCtrl+LPS	95.05	95.05	96.04	97.03	97.03	96.04	0.99
	Ad-shMCT1+LPS	94.06	94.06	97.03	98.02	98.02	96.37	2.06
sLA-MHCII	control	31.68	31.68	34.65	38.61	38.61	34.98	3.48
	LPS	62.38	62.38	64.36	67.33	67.33	64.69	2.49
	Ad-shCtrl+LPS	62.38	62.38	64.36	68.32	68.32	65.02	3.02
	Ad-shMCT1+LPS	84.16	84.16	87.13	88.12	88.12	86.47	2.06

SD – standard deviation; Q1 – 1<sup>st</sup> quartile; Q3 – 3<sup>rd</sup> quartile; LPS – lipopolysaccharide; sLA – sodium lactate; MHCII – major histocompatibility complex class II; Ad-shMCT1 – mouse SLC16A1 (MCT1) shRNA silencing adenovirus; PBS – phosphate-buffered saline.



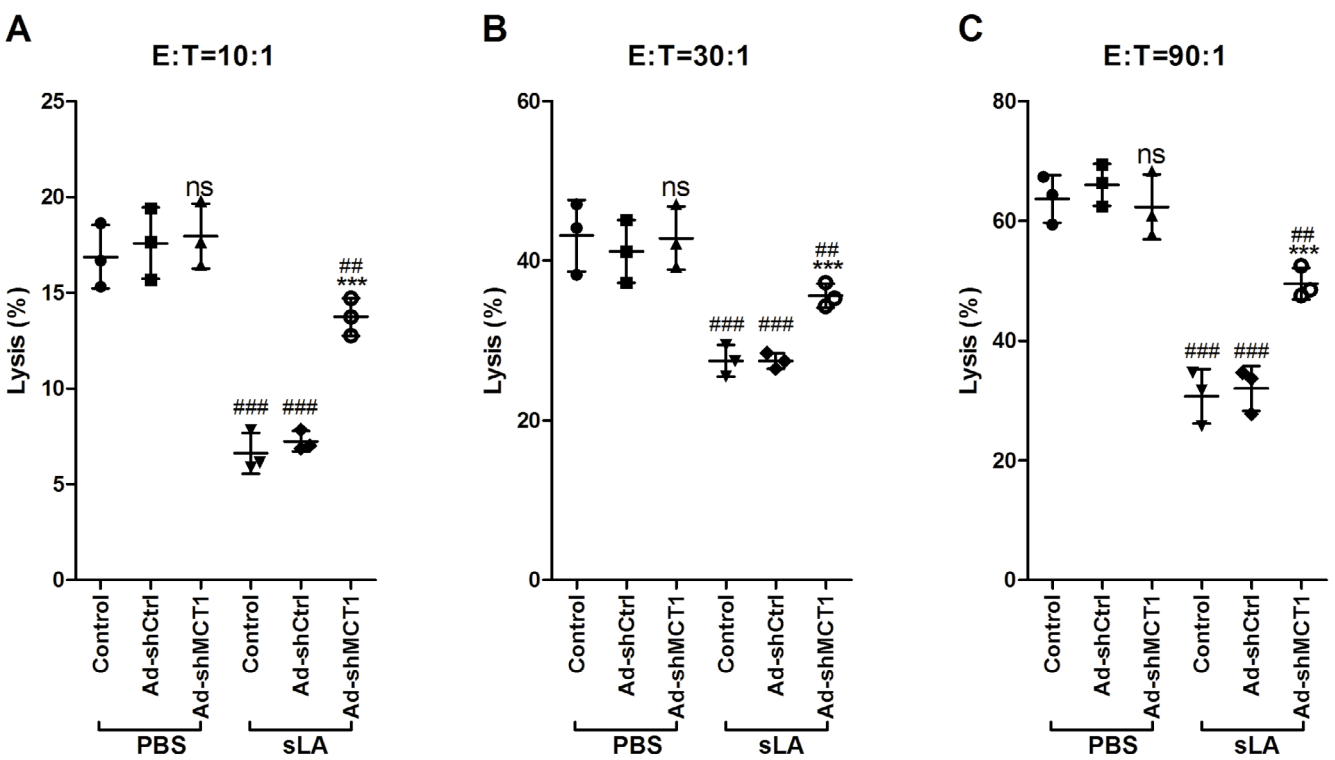
**Fig. 3.** Secretion of cytokine from dendritic cells (DCs). Dendritic cells were first infected with Ad-shCtrl and mouse SLC16A1 (MCT1) shRNA silencing (Ad-shMCT1) adenoviruses for 24 h, then co-cultured with 50 mM of sodium lactate (sLA) or phosphate-buffered saline (PBS) for 48 h, and finally stimulated with 1 μg/mL lipopolysaccharide (LPS) for 24 h. The content of interleukin (IL)-6, IL-12 p70 and tumor necrosis factor alpha (TNF-α) in the DC culture medium was detected by enzyme-linked immunosorbent assay (ELISA) kit. The column height represents the average value, and the error bar represents the standard deviation. The p-value was calculated by Tukey's honestly significant difference (HSD) test following the analysis of variance (ANOVA) testing between multiple groups or by Student's t-test (PBS compared to sLA treatment)

ns  $p > 0.05$ , \*\*\*  $p < 0.001$  compared to the Ad-shCtrl+LPS group; #  $p < 0.05$ , ##  $p < 0.01$  and ###  $p < 0.001$  compared to the same treatment in the PBS group.

**Table 3.** Statistics for Fig. 3 (n = 3)

Variable	Minimal	Q1	Median	Q3	Maximal	Mean	SD
Normal IL-6	control	1.98	1.98	6.93	10.89	6.60	4.46
	Ad-shCtrl	2.97	2.97	6.93	14.85	8.25	6.05
	Ad-shMCT1	4.95	4.95	7.92	12.87	8.58	4.00
PBS IL-6	control	235.60	235.60	242.60	262.40	246.90	13.88
	Ad-shCtrl	235.60	235.60	242.60	262.40	246.90	13.88
	Ad-shMCT1	239.60	239.60	252.50	256.40	249.50	8.81
sLA IL-6	control	133.70	133.70	154.50	166.30	151.50	16.54
	Ad-shCtrl	130.70	130.70	147.50	170.30	149.50	19.88
	Ad-shMCT1	216.80	216.80	222.80	229.70	223.10	6.44
Normal IL-12 p70	control	2.97	2.97	4.95	5.94	4.62	1.51
	Ad-shCtrl	2.97	2.97	3.96	5.94	4.29	1.51
	Ad-shMCT1	1.98	1.98	3.96	7.92	4.62	3.03
PBS IL-12 p70	control	137.60	137.60	143.60	161.40	147.50	12.37
	Ad-shCtrl	136.60	136.60	140.60	158.40	145.20	11.61
	Ad-shMCT1	137.60	137.60	153.50	158.40	149.80	10.87
sLA IL-12 p70	control	61.39	61.39	67.33	81.19	69.97	10.16
	Ad-shCtrl	64.36	64.36	72.28	74.26	70.30	5.24
	Ad-shMCT1	108.90	108.90	120.80	126.70	118.80	9.07
Normal TNF-α	control	2.94	2.94	4.90	8.82	5.55	2.99
	Ad-shCtrl	4.90	4.90	5.88	7.84	6.21	1.50
	Ad-shMCT1	2.94	2.94	3.92	8.82	5.23	3.15
PBS TNF-α	control	259.80	259.80	277.50	293.10	276.80	16.68
	Ad-shCtrl	262.80	262.80	274.50	292.20	276.50	14.80
	Ad-shMCT1	248.00	248.00	287.30	299.00	278.10	26.69
sLA TNF-α	control	150.00	150.00	161.80	168.60	160.10	9.42
	Ad-shCtrl	152.00	152.00	155.90	176.50	161.40	13.17
	Ad-shMCT1	235.30	235.30	248.00	262.80	248.70	13.74

SD – standard deviation; Q1 – 1<sup>st</sup> quartile; Q3 – 3<sup>rd</sup> quartile; sLA – sodium lactate; IL – interleukin; TNF-α – tumor necrosis factor alpha; Ad-shMCT1 – mouse SLC16A1 (MCT1) shRNA silencing adenovirus; PBS – phosphate-buffered saline.



**Fig. 4.** Lysis rate of U14 cells by dendritic cell (DC)-induced cytotoxic T lymphocytes (CTLs). Different CTLs were co-cultured with U14 at different seeding ratios of 10:1 (A), 30:1 (B) and 90:1 (C) for 72 h, and then the cell viability was detected using a Cell Counting Kit-8 (CCK-8). Control conditions were the activity of U14 cells cultured alone. The column height represents the average value, and the error bar represents the standard deviation. The p-value was calculated by Tukey's honestly significant difference (HSD) test following the analysis of variance (ANOVA) between multiple groups or by Student's t-test (phosphate-buffered saline (PBS) compared to sodium lactate (sLA) treatment)

E – effector (DC-induced CTLs); T – target (U14 cells); ns  $p > 0.05$ , \*\*\*  $p < 0.001$  compared to the Ad-shCtrl group; #  $p < 0.05$ , ##  $p < 0.01$  and ###  $p < 0.001$  compared to the same treatment in the PBS group.

**Table 4.** Statistics for Fig. 4 (n = 3)

Variable		Minimal	Q1	Median	Q3	Maximal	Mean	SD
10:1 PBS	control	15.32	15.32	16.67	18.63	18.63	16.87	1.66
	Ad-shCtrl	15.69	15.69	17.65	19.41	19.41	17.58	1.86
	Ad-shMCT1	16.44	16.44	17.65	19.79	19.79	17.96	1.70
10:1 sLA	control	5.88	5.88	6.13	7.84	7.84	6.62	1.07
	Ad-shCtrl	6.86	6.86	7.02	7.84	7.84	7.24	0.53
	Ad-shMCT1	12.75	12.75	13.73	14.71	14.71	13.73	0.98
30:1 PBS	control	38.24	38.24	44.12	47.06	47.06	43.14	4.49
	Ad-shCtrl	37.25	37.25	41.18	45.10	45.10	41.18	3.93
	Ad-shMCT1	39.22	39.22	42.16	47.06	47.06	42.81	3.96
30:1 sLA	control	25.49	25.49	27.45	29.41	29.41	27.45	1.96
	Ad-shCtrl	26.47	26.47	27.45	28.43	28.43	27.45	0.98
	Ad-shMCT1	34.31	34.31	35.29	37.25	37.25	35.62	1.50
90:1 PBS	control	59.41	59.41	64.36	67.33	67.33	63.70	4.00
	Ad-shCtrl	62.38	62.38	66.34	69.37	69.37	66.03	3.51
	Ad-shMCT1	57.72	57.72	60.89	68.32	68.32	62.31	5.44
90:1 sLA	control	25.74	25.74	31.68	34.65	34.65	30.69	4.54
	Ad-shCtrl	27.72	27.72	33.66	34.65	34.65	32.01	3.75
	Ad-shMCT1	47.52	47.52	48.51	52.48	52.48	49.50	2.63

SD – standard deviation; Q1 – 1<sup>st</sup> quartile; Q3 – 3<sup>rd</sup> quartile; sLA – sodium lactate; Ad-shMCT1 – mouse SLC16A1 (MCT1) shRNA silencing adenovirus; PBS – phosphate-buffered saline.

In the absence of sLA exposure, MCT1 silencing did not affect the secretion of IL-6, IL-12 p70 and TNF- $\alpha$  from DCs. However, the exposure of DCs to sLA and LPS decreased the levels of these cytokines.

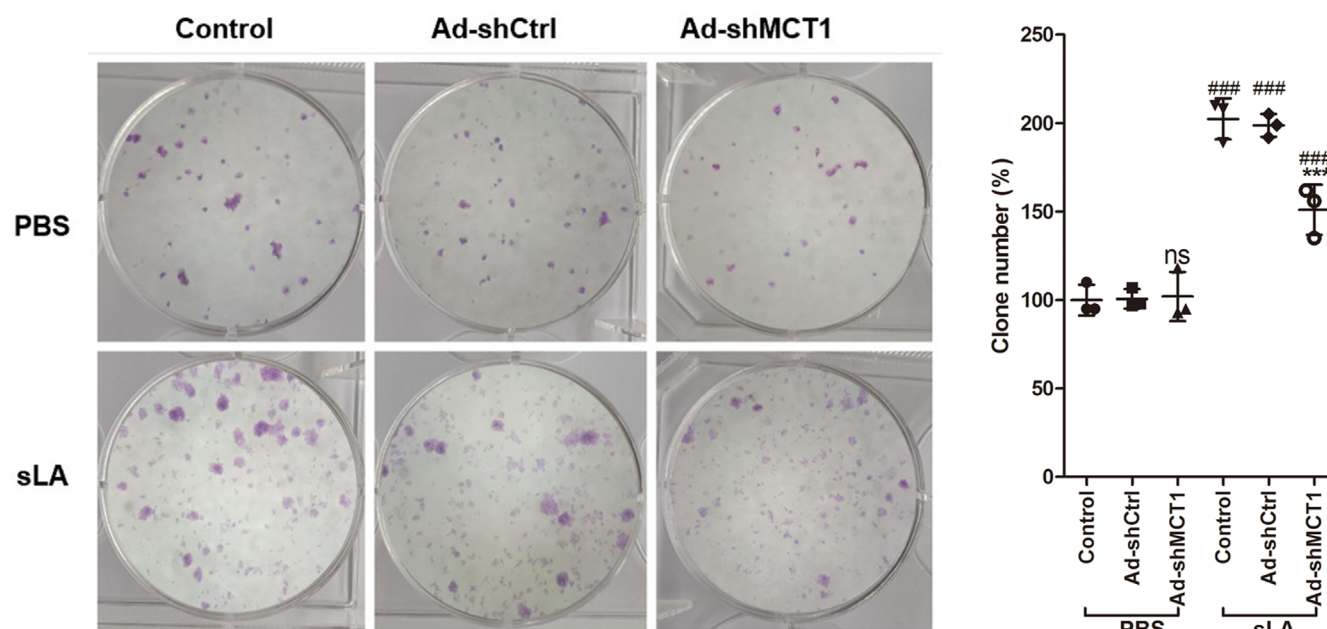
### Impact of *MCT1* gene silencing on the immune effect of DCs

To analyze the toxicity effect of DC-induced CTLs on cervical cancer cells, we generated CTLs (defined as DC-induced CTLs) by co-culturing DCs and T cells for 1 week, and then co-culturing the obtained CTLs with U14 cells. In DC-induced CTLs without sLA exposure, the lysis rate of U14 cells was independent of whether MCT1 was silenced ( $p < 0.05$ , HSD test following ANOVA, Fig. 4 and Table 4). However, DC-induced CTLs with sLA exposure and MCT1 silencing significantly increased the lysis rate of U14 cells ( $p < 0.05$ , HSD test following ANOVA testing,

Fig. 4 and Table 4). Simultaneously, in a DC-induced CTL and U14 cell co-culture system, the MCT1 silencing without sLA exposure did not affect the number of U14 clones, but it decreased the U14 cell clone number ( $p < 0.05$ , HSD test following ANOVA testing, Fig. 5 and Table 5). Consistently, in vivo, MCT1 silencing without sLA exposure did not affect the weight of U14 xenograft, while xenografts demonstrated significantly decreased weight with MCT1 silencing and sLA exposure ( $p < 0.05$ , HSD test following ANOVA testing, Fig. 6 and Table 6).

## Discussion

For decades, lactate was considered a metabolic waste product. However, in recent years, it has been reported that the output of lactic acid from the glycolysis of tumor cells can promote the proliferation of tumor cells in the tumor



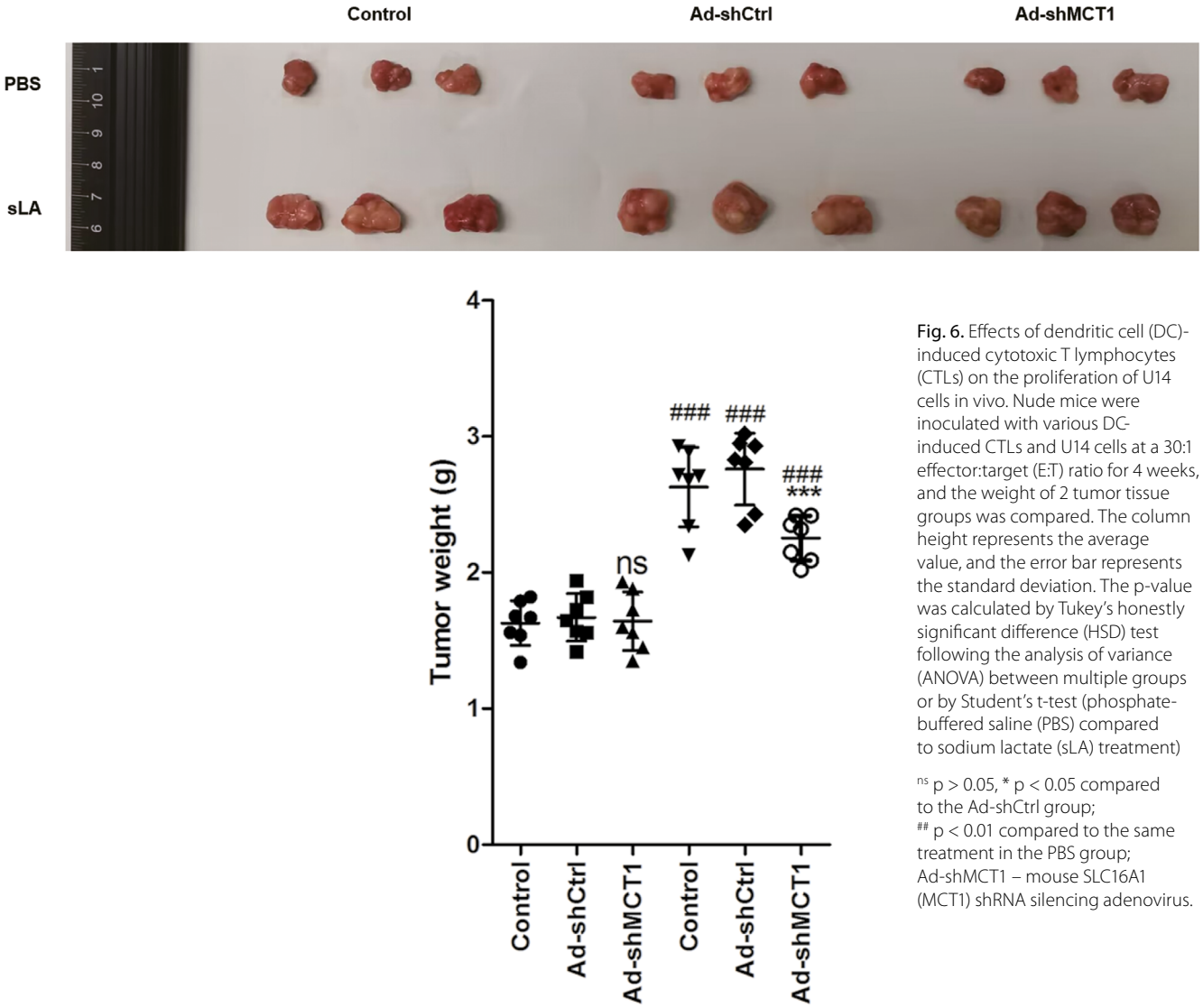
**Fig. 5.** Effects of dendritic cell (DC)-induced cytotoxic T lymphocytes (CTLs) on the proliferation of U14 cells in vitro. Different CTLs were co-cultured with U14 cells at 30:1 effector:target (E:T) ratio for 2 weeks, and then the colony numbers were counted for comparison. The column height represents the average value, and the error bar is the standard deviation. The p-value was calculated by Tukey's honestly significant difference (HSD) test following the analysis of variance (ANOVA) between multiple groups or by Student's t-test (phosphate-buffered saline (PBS) compared to sodium lactate (sLA) treatment)

<sup>ns</sup>  $p > 0.05$ , <sup>\*\*\*</sup>  $p < 0.001$  compared to the Ad-shCtrl group; <sup>###</sup>  $p < 0.001$  compared to the same treatment in the PBS group; Ad-shMCT1 – mouse SLC16A1 (MCT1) shRNA silencing adenovirus.

**Table 5.** Statistics for Fig. 5 ( $n = 3$ )

Variable		Minimal	Q1	Median	Q3	Maximal	Mean	SD
PBS	control	95	95	95	110	110	100	8.66
	Ad-shCtrl	97	97	98	107	107	100.7	5.508
	Ad-shMCT1	93	93	95	118	118	102	13.89
sLA	control	189	189	208	210	210	202.3	11.59
	Ad-shCtrl	192	192	199	205	205	198.7	6.506
	Ad-shMCT1	135	135	156	162	162	151	14.18

SD – standard deviation; Q1 – 1<sup>st</sup> quartile; Q3 – 3<sup>rd</sup> quartile; sLA – sodium lactate; Ad-shMCT1 – mouse SLC16A1 (MCT1) shRNA silencing adenovirus; PBS – phosphate-buffered saline.



**Fig. 6.** Effects of dendritic cell (DC)-induced cytotoxic T lymphocytes (CTLs) on the proliferation of U14 cells in vivo. Nude mice were inoculated with various DC-induced CTLs and U14 cells at a 30:1 effector:target (E:T) ratio for 4 weeks, and the weight of 2 tumor tissue groups was compared. The column height represents the average value, and the error bar represents the standard deviation. The p-value was calculated by Tukey's honestly significant difference (HSD) test following the analysis of variance (ANOVA) between multiple groups or by Student's t-test (phosphate-buffered saline (PBS) compared to sodium lactate (sLA) treatment)

<sup>ns</sup>  $p > 0.05$ , \*  $p < 0.05$  compared to the Ad-shCtrl group;  
<sup>##</sup>  $p < 0.01$  compared to the same treatment in the PBS group;  
Ad-shMCT1 – mouse SLC16A1 (MCT1) shRNA silencing adenovirus.

**Table 6.** Statistics for Fig. 6 (n = 3)

Variable		Minimal	Q1	Median	Q3	Maximal	Mean	SD
PBS	control	1.34	1.54	1.67	1.79	1.82	1.63	0.16
	Ad-shCtrl	1.42	1.56	1.65	1.82	1.94	1.67	0.18
	Ad-shMCT1	1.35	1.45	1.60	1.88	1.93	1.64	0.22
sLA	control	2.13	2.34	2.71	2.89	2.93	2.63	0.29
	Ad-shCtrl	2.35	2.43	2.83	2.95	3.02	2.76	0.26
	Ad-shMCT1	2.02	2.09	2.32	2.42	2.42	2.25	0.16

SD – standard deviation; Q1 – 1<sup>st</sup> quartile; Q3 – 3<sup>rd</sup> quartile; sLA – sodium lactate; Ad-shMCT1 – mouse SLC16A1 (MCT1) shRNA silencing adenovirus; PBS – phosphate-buffered saline.

microenvironment. Moreover, it aids in immune tolerance and helps tumor cells escape detection by immune cells.<sup>21–23</sup> The Warburg Effect of cancer cells makes them more inclined to use glycolysis for energy, and the accompanying lactic acid is a product of their metabolism. Importantly, lactate exported by cancer cells into the tumor microenvironment promotes tumor cell proliferation, metastasis, angiogenesis, and immune tolerance.<sup>9,10</sup> It has been reported that lactate is a potent inhibitor of T cell and

NK cell survival and function, modulating the phenotype of DCs and macrophages.<sup>11–13</sup> A recent study reported that AZD3965, an MCT1 inhibitor, could reverse the immunosuppressive micro-environment of solid tumors, thereby improving the safety and anti-tumor efficacy of cancer immunotherapy.<sup>24</sup> Hence, the inhibition of MCT1 can be viewed as a new strategy for tumor immunotherapy.

In this study, we silenced the MCT1 expression in BM-derived DCs by adenovirus infection and found that MCT1

silencing affected the phenotype and cytokine secretion of BM-derived DCs, although only when also exposed to sLA. Specifically, MCT1 silencing upregulated the expression of CD1a, CD80, CD83, CD86, and MHCII, and the secretion of IL-6, IL-12 p70 and TNF- $\alpha$  in BM-derived DCs after sLA exposure and LPS challenge. The MCT1 is a member of the monocarboxylate transporter family, which plays an essential regulatory role in glycolysis by mediating the transmembrane transport of lactate.<sup>25,26</sup> It has been reported that MCT1 is upregulated in cervical cancer tissues and contributes to disease progression by promoting the proliferation, migration and angiogenesis of cervical cancer cells. Its mechanism is thought to be related to the regulation of lactate metabolism in the tumor microenvironment.<sup>27–29</sup>

Furthermore, lactate that is accumulated in the tumor microenvironment is transported from extracellular to intracellular microenvironment via MCT1 to regulate its phenotypic changes, ultimately resulting in immunosuppression.<sup>13,18</sup> This is considered an essential measure of immune evasion of tumor cells. Sangsuwan et al. revealed that lactate exposure reduces the expression of CD11c, CD80, CD86, and MCHII, and results in a decreased secretion of IL-12 in DCs, which is consistent with the results of the present study.<sup>13</sup> More importantly, our study found that silencing MCT1 attenuated these changes induced by lactate exposure in DCs, providing a new strategy for tumor immunotherapy.

A robust immune response against tumors depends on several factors, such as the degree of maturation and activation of DCs, their ability to capture, process and present exogenous antigens, as well as their transport to secondary lymphoid organs and the tissue types from which they arise.<sup>30,31</sup> Dendritic cells are critical for initiating anti-tumor immunity, as they activate various immune cells, including T cells, to establish anti-tumor resistant barriers. In this study, we demonstrated that we could induce CTLs by co-culturing DCs and splenic T cells. Our findings showed that CTLs derived from sLA-exposed DCs were less effective against U14 cells than those derived from DCs not exposed to sLA. However, MCT1 silencing significantly increased the toxicity of sLA-exposed DC-derived CTLs to U14 cells both in vivo and in vitro. The results suggest that lactate exposure reduced the antigen-presenting capacity of DCs, but MCT1 silencing could attenuate the effects of lactate exposure. Under physiological conditions, DCs often exhibit an immature phenotype in vivo, characterized by low surface levels of MHCII and costimulatory molecules, and induce suboptimal T cell priming, often resulting in T cell anergy or tolerance.<sup>32–34</sup> Dendritic cells matured and activated by antigens were found to highly express MHCII and costimulatory molecules (CD80, CD83 and CD86), which induced potent T cell activation and effector differentiation.<sup>35,36</sup> However, lactate-mediated signaling has been shown to hinder the maturation, activation and antigen presentation of DCs, resulting in widespread

immunosuppression.<sup>37</sup> The MCT1 is a crucial protein involved in the signaling pathways of lactate in DCs, and conversely, lactate has been shown to regulate the expression of MCT1. Therefore, silencing of MCT1 could theoretically attenuate the functional and phenotypic changes of DCs induced by lactate exposure. Our findings provide evidence to support this hypothesis.

## Limitations

The present study demonstrated that *MCT1* gene silencing attenuated lactate exposure and decreased dendritic cell immunity against cervical cancer cells both in vitro and in vivo. However, this study did not further investigate the specific molecular mechanism of the changes caused by MCT1 silencing.

## Conclusions

Lactate exposure reduces the immunological effect of DCs on cervical cancer cells. However, this effect can be mitigated by *MCT1* gene silencing, thereby reducing the impact of lactate exposure on DCs.

## Data availability

The dataset used and/or analyzed during the current study is available from the corresponding author upon reasonable request.

## Supplementary data


The supplementary materials are available at <https://doi.org/10.5281/zenodo.8281170>. The package contains the following files:

- Supplementary Table 1. Statistics for Fig. 1.
- Supplementary Table 2. Statistics for Fig. 2.
- Supplementary Table 3. Statistics for Fig. 3.
- Supplementary Table 4. Statistics for Fig. 4.
- Supplementary Table 5. Statistics for Fig. 5.
- Supplementary Table 6. Statistics for Fig. 6.
- Supplementary Table 7. Statistics for Supplementary Fig. 1.

Supplementary Fig. 1. Cube maps for the levels of DC marker expression after sLA exposure and LPS challenge. Dendritic cells were first infected with Ad-shCtrl and Ad-shMCT1 adenoviruses for 24 h, then co-cultured with 50 mM of sLA or PBS for 48 h, and finally stimulated with 1  $\mu$ g/mL LPS for 24 h. Flow cytometry was used to detect the expression of CD1a (A), CD80 (B), CD83 (C), CD86 (D), and MHCII (E) in DCs. The column height represents the average value, and the bar of the column is the standard deviation. The p-value was calculated by Tukey's honestly significant difference (HSD) test following analysis of variance (ANOVA) between multiple groups or by Student's t-test (PBS compared to sLA treatment).

## ORCID iDs

Xiaoxin Sui  <https://orcid.org/0009-0009-4863-0945>

Xiaowei Xi  <https://orcid.org/0000-0001-7306-3254>

## References

- Gardner A, De Mingo Pulido Á, Ruffell B. Dendritic cells and their role in immunotherapy. *Front Immunol*. 2020;11:924. doi:10.3389/fimmu.2020.00924
- Collin M, Bigley V. Human dendritic cell subsets: An update. *Immunology*. 2018;154(1):3–20. doi:10.1111/imm.12888
- Lee YS, Radford KJ. The role of dendritic cells in cancer. *Int Rev Cell Mol Biol*. 2019;348:123–178. doi:10.1016/bs.ircmb.2019.07.006
- Patente TA, Pinho MP, Oliveira AA, Evangelista GCM, Bergami-Santos PC, Barbuto JAM. Human dendritic cells: Their heterogeneity and clinical application potential in cancer immunotherapy. *Front Immunol*. 2019;9:3176. doi:10.3389/fimmu.2018.03176
- Enamorado M, Khoulil SC, Iborra S, Sancho D. Genealogy, dendritic cell priming, and differentiation of tissue-resident memory CD8<sup>+</sup> T cells. *Front Immunol*. 2018;9:1751. doi:10.3389/fimmu.2018.01751
- Fu C, Jiang A. Dendritic cells and CD8 T cell immunity in tumor microenvironment. *Front Immunol*. 2018;9:3059. doi:10.3389/fimmu.2018.03059
- Hou B, Tang Y, Li W, Zeng Q, Chang D. Efficiency of CAR-T therapy for treatment of solid tumor in clinical trials: A meta-analysis. *Dis Markers*. 2019;2019:3425291. doi:10.1155/2019/3425291
- Akhoundi M, Mohammadi M, Sahraei SS, Sheykhasan M, Fayazi N. CAR T cell therapy as a promising approach in cancer immunotherapy: Challenges and opportunities. *Cell Oncol*. 2021;44(3):495–523. doi:10.1007/s13402-021-00593-1
- Vaupel P, Schmidberger H, Mayer A. The Warburg Effect: Essential part of metabolic reprogramming and central contributor to cancer progression. *Int J Radiat Biol*. 2019;95(7):912–919. doi:10.1080/09553002.2019.1589653
- San-Millán I, Brooks GA. Reexamining cancer metabolism: Lactate production for carcinogenesis could be the purpose and explanation of the Warburg Effect. *Carcinogenesis*. 2016;38(2):119–133. doi:10.1093/carcin/bgw127
- Brand A, Singer K, Koehl GE, et al. LDHA-associated lactic acid production blunts tumor immunosurveillance by T and NK cells. *Cell Metab*. 2016;24(5):657–671. doi:10.1016/j.cmet.2016.08.011
- Fischer K, Hoffmann P, Voelkl S, et al. Inhibitory effect of tumor cell-derived lactic acid on human T cells. *Blood*. 2007;109(9):3812–3819. doi:10.1182/blood-2006-07-035972
- Sangsuwan R, Thuamsang B, Pacifici N, et al. Lactate exposure promotes immunosuppressive phenotypes in innate immune cells. *Cel Mol Bioeng*. 2020;13(5):541–557. doi:10.1007/s12195-020-00652-x
- Halestrap AP. The SLC16 gene family: Structure, role and regulation in health and disease. *Mol Aspects Med*. 2013;34(2–3):337–349. doi:10.1016/j.mam.2012.05.003
- Sun X, Wang M, Wang M, et al. Role of proton-coupled monocarboxylate transporters in cancer: From metabolic crosstalk to therapeutic potential. *Front Cell Dev Biol*. 2020;8:651. doi:10.3389/fcell.2020.00651
- Fiaschi T, Marini A, Giannoni E, et al. Reciprocal metabolic reprogramming through lactate shuttle coordinately influences tumor–stroma interplay. *Cancer Res*. 2012;72(19):5130–5140. doi:10.1158/0008-5472.CAN-12-1949
- Doherty JR, Cleveland JL. Targeting lactate metabolism for cancer therapeutics. *J Clin Invest*. 2013;123(9):3685–3692. doi:10.1172/JCI69741
- Manoharan I, Prasad PD, Thangaraju M, Manicassamy S. Lactate-dependent regulation of immune responses by dendritic cells and macrophages. *Front Immunol*. 2021;12:691134. doi:10.3389/fimmu.2021.691134
- National Research Council (U.S.), Institute for Laboratory Animal Research (U.S.), National Academies Press (U.S.), eds. *Guide for the Care and Use of Laboratory Animals*. 8<sup>th</sup> ed. Washington, D.C, USA: National Academies Press; 2011. ISBN:978-0-309-15400-0/978-0-309-15401-7.
- Zhu Y, Zheng Y, Mei L, et al. Enhanced immunotherapeutic effect of modified HPV16 E7-pulsed dendritic cell vaccine by an adeno-shRNA-SOCS1 virus. *Int J Oncol*. 2013;43(4):1151–1159. doi:10.3892/ijo.2013.2027
- Abbaszadeh Z, Çeşmeli S, Biray Avcı Ç. Crucial players in glycolysis: Cancer progress. *Gene*. 2020;726:144158. doi:10.1016/j.gene.2019.144158
- Chen AN, Luo Y, Yang YH, et al. Lactylation, a novel metabolic reprogramming code: Current status and prospects. *Front Immunol*. 2021;12:688910. doi:10.3389/fimmu.2021.688910
- Brown TP, Ganapathy V. Lactate/GPR81 signaling and proton motive force in cancer: Role in angiogenesis, immune escape, nutrition, and Warburg phenomenon. *Pharmacol Ther*. 2020;206:107451. doi:10.1016/j.pharmthera.2019.107451
- Huang T, Feng Q, Wang Z, et al. Tumor-targeted inhibition of monocarboxylate transporter 1 improves T-cell immunotherapy of solid tumors. *Adv Healthcare Mater*. 2021;10(4):2000549. doi:10.1002/adhm.202000549
- Payen VL, Mina E, Van Hée VF, Porporato PE, Sonveaux P. Monocarboxylate transporters in cancer. *Mol Metab*. 2020;33:48–66. doi:10.1016/j.molmet.2019.07.006
- Zhao Y, Li M, Yao X, et al. HCAR1/MCT1 regulates tumor ferroptosis through the lactate-mediated AMPK-SCD1 activity and its therapeutic implications. *Cell Rep*. 2020;33(10):108487. doi:10.1016/j.celrep.2020.108487
- Pinheiro C, Garcia EA, Morais-Santos F, et al. Reprogramming energy metabolism and inducing angiogenesis: Co-expression of monocarboxylate transporters with VEGF family members in cervical adenocarcinomas. *BMC Cancer*. 2015;15(1):835. doi:10.1186/s12885-015-1842-4
- Silva LS, Gonçalves LG, Silva F, et al. STAT3/FOXO1 and MCT1 drive uterine cervix carcinoma fitness to a lactate-rich microenvironment. *Tumor Biol*. 2016;37(4):5385–5395. doi:10.1007/s13277-015-4385-z
- Payen VL, Hsu MY, Räddecke KS, et al. Monocarboxylate transporter MCT1 promotes tumor metastasis independently of its activity as a lactate transporter. *Cancer Res*. 2017;77(20):5591–5601. doi:10.1158/0008-5472.CAN-17-0764
- Wculek SK, Cueto FJ, Mujal AM, Melero I, Krummel MF, Sancho D. Dendritic cells in cancer immunology and immunotherapy. *Nat Rev Immunol*. 2020;20(1):7–24. doi:10.1038/s41577-019-0210-z
- Nava S, Lisini D, Frigerio S, Bersano A. Dendritic cells and cancer immunotherapy: The adjuvant effect. *Int J Mol Sci*. 2021;22(22):12339. doi:10.3390/ijms222212339
- Jang JE, Hajdu CH, Liot C, Miller G, Dustin ML, Bar-Sagi D. Cross-talk between regulatory T cells and tumor-associated dendritic cells negates anti-tumor immunity in pancreatic cancer. *Cell Rep*. 2017;20(3):558–571. doi:10.1016/j.celrep.2017.06.062
- Davison GM. Dendritic cells, T-cells and their possible role in the treatment of leukaemia and lymphoma. *Transfus Apher Sci*. 2010;42(2):189–192. doi:10.1016/j.transci.2010.01.018
- Garris CS, Luke JJ. Dendritic cells, the T-cell-inflamed tumor microenvironment, and immunotherapy treatment response. *Clin Cancer Res*. 2020;26(15):3901–3907. doi:10.1158/1078-0432.CCR-19-1321
- Shevchenko J, Khristin A, Kurilin V, et al. Autologous dendritic cells and activated cytotoxic T-cells as combination therapy for breast cancer. *Oncol Rep*. 2019;43(2):671–680. doi:10.3892/or.2019.7435
- Gajewski TF, Schreiber H, Fu YX. Innate and adaptive immune cells in the tumor microenvironment. *Nat Immunol*. 2013;14(10):1014–1022. doi:10.1038/ni.2703
- Srinivasan S, Babensee J. Dendritic cells support a proliferative antigen-specific T-cell response in the presence of poly(lactic-co-glycolic acid). *J Biomed Mater Res*. 2021;109(11):2269–2279. doi:10.1002/jbm.a.37211