Therapeutic and anti-metastatic effect of CIKs on cervical cancer enhanced by modified HPV16 E7-pulsed DCs with SOCS1-knockdown

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Abstract: Cervical cancer is the second most frequent cancer and the second most common cause of cancer-related deaths among women worldwide. In this study, a modified HPV16 E7 (HPV16mE7) with reduced transformation activity and enhanced antigenicity was employed to load DCs with silenced suppressor of cytokine signaling 1 (SOCS1) gene. The prepared DCs were co-cultured with peripheral blood mononuclear cells (PBMCs)-derived CIKs. The resulted DCCIKs were then transfused into mouse models bearing tumor of HPV16 E6/E7 expressing SiHa cells for the in vitro/in vivo antitumor activity assay. The expression levels of IL-12, IL-6, TNF-α and IFN-γ were also evaluated. The survival days and the tumor volume from different treatment groups were measured as well as the in vivo heterotopic tumor metastasis rate from the liver to the lung established in the mouse models. The data demonstrated that the xenografted tumor mice treated with Ad-shSOCS1-DCCIKs exhibited better immunotherapeutic and antimetastatic effect. This method may provide a new strategy for the development of safe and effective immunotherapy for cervical cancer.

Keywords: Cervical cancer, HPV, DC, CIKs, SOCS1, RNAi, metastasis

Introduction

Cervical cancer is one of the most common gynecologic malignant tumors. Molecular, clinical and epidemiological researches identify human papillomavirus (HPV) infection is responsible for nearly all cervical cancer cases [1, 2]. HPV16 and HPV18 are associated with 70% oncogenesis cause of cervical cancer cases, and therefore are considered as “high risk” (HR) viruses [3-6]. Encoded by these two viruses, the E6 and E7 oncoproteins are crucial for the viral life cycle in the transformation and maintenance of the malignant phenotype of cervical cancer [7]. Various immunotherapeutic strategies against E6 and E7 were developed, however they have largely proven ineffective in treating HPV-induced cervical neoplasia, mainly attributing to the patients’ severe cellular immune function defects such as the activated immune escape and tolerance mechanisms employed by tumors and virally infected cells [8, 9].

Therefore, improving the cervical cancer patients’ cellular immunity functions is essential to improve the cure rate, prolong the survival time and inhibit tumor recurrence or metastasis. In recent years, the applications of adoptive cellular immunotherapy such as CIKs, CAR T cells, DC, DCCIKs, natural killer (NK) cells, tumor-infiltrating lymphocytes (TILs), and cytotoxic T lymphocytes (CTLs) have been constantly expanding and gradually accepted [10-13].

Possessing the properties of strong antitumor activity of T lymphocytes and a non-major histocompatibility complex (MHC)-restricted tumoricidal effect, CIK cells generated from PBMCs and ex vivo cultured in the presence of a variety
of cytokines have become the preferred type of cells used in clinic practice [10, 12, 13]. When co-cultured with DCs which can generate antigen-specific immune responses, CIKs activated by DCs (DCCIKs) achieve the advantages of fast proliferation, high cytotoxicity, and a broad tumor-killing spectrum [11].

DCs which are the most professional antigen-presenting cells (APC) are considered at the centre of the immune system because of their ability to regulate both immune tolerance and immunity. And the generation of protective anti-tumor immunity depends on the presentation of tumor antigens by DCs [11, 14]. Activated (mature), antigen-loaded and ex vivo cultured DCs can induce the differentiation of antigen-specific T cells into effector T cells which can reduce the tumor mass specifically as well as control tumor relapse, in contrast to non-activated (immature) DCs which can present self-antigens to T cells, leading to immune tolerance by generating suppressor T cells or T cell deletion [14-16]. As the presentation of tumor antigens by DCs play a decisive role in generating protective anti-tumor immunity in mouse models [17, 18], therefore, the activation of the full immune stimulatory potential of DCs to achieve an effective immunity provides a promising treatment against tumor.

As an inducible negative feedback inhibitor of the Jak/STAT signal pathway [14] and negative regulator of various cytokines such as interferon (IFN)-γ, IL-2, IL-6, IL-7, IL-12, and IL-15 in DCs, suppressor of cytokine signaling 1 (SOCS1) has been implicated in exerting potent immunosuppressive effect on blocking the constitutive activation of the immune response [19]. DCs derived from SOCS1-/- transgenic mice were found hyper-responsive to LPS and IFN-γ, triggering an allogeneic T-cell expansion [20, 21].

In this study, HPV16mE7-loaded DCs with SOCS1-knock down were co-cultured with CIK cells prepared from human PBMCs. The generated DCCIKs were infused into mouse models bearing tumor of viral HPV16 E6E7 expressing SiHa cells. The survival days and the tumor volume from different treatment groups were investigated as well as the in vivo heterotopic tumor metastasis rate from liver to lung. The results demonstrated that the xenografted tumor mice treated with Ad-shSOCS1-DCCIKs exhibited better immunotherapeutic and anti-metastatic effect.

Materials and methods

Cells and reagents

SiHa and HEK293 cell lines were purchased from American Type Culture Collection (ATCC). Cells were maintained in the DMEM or RPMI-1640 culture media (Gibco, Life Technologies, US) supplemented with 10% (v/v) fetal bovine serum (FBS) (Invitrogen, Carlsbad, CA) and 2 mM l-glutamine (Invitrogen, Carlsbad, CA) and in a humidified atmosphere containing 5% CO₂ at 37°C. PBMCs were generated from healthy donors whose informed consent was obtained from the participants in accordance with the study protocols approved by the Institutional Review Board of the Hospital Authority of Wuhan. rhIL-2 and rhGM-CSF were acquired from R&D Inc. (USA). rhIL-4, rhIFN-γ, and TNF-α were purchased from Peprotech Inc. Normal human AB serum were sourced from (Gibco, Life Technologies, USA). Lymphocyte separation medium Ficoll was purchased from GE Healthcare (USA). Mouse anti-human CD3 (FITC labeled) monoclonal antibody, mouse anti-human CD8 (PE labeled) monoclonal antibody, and mouse anti-human CD56 (PE labeled) monoclonal antibody were purchased from eBioscience Co, Ltd. Mouse anti-human CD80-PE monoclonal antibody, mouse anti-human CD83-PE monoclonal antibody, mouse anti-human CD86-PE monoclonal antibody, mouse anti-human CD40-FITC monoclonal antibody, and mouse antihuman CD1a-FITC monoclonal antibody were all products of Santa Cruz Co, Ltd. Female BALB/c nude mice (5-8 weeks of age, weighing 18-22 g) raised under SPF circumstance were purchased from the Shanghai Laboratory Animal Center, Chinese Academy of Science. All animal studies were conducted in accordance with the Guide for the Care and Use of Laboratory Animals.

Recombinant adenovirus production

Primers used for shRNA-SOCS1 are as follows: shRNA-SOCS1 (F): 5’-GATCCCCCTAATCCGAGTTCC-TTTCTTCAAGAGGAAAGGAAACTGAGGTTTTAGTTTTGG-3’ (BamHI and EcoRI); (R): 5’-AAT-TCATAGAACTACCTAGGTTCCCTTCCCTCCCTCTTGGGAAAGGAAACTGAGGTAGG3’; shRNA-Control (F): 5’-GATCTCACACCTCTAGAAAGAGTA-
GATTGTACTACAAAAAGTGACTATGGTTTTTG-3' (BamH I and EcoR I); (R): 5'-AATTCAAAAAACA-
TAGTACTTTTGTGAGTACAATCTACTCTTTCTA-
GGAGGTTGTGAG. The shRNA-SOCS1 and shR-
NA-Control were cloned into pShuttle-2 vector (BD Clontech), and then inserted into the repli-
cation-deficient pAdeno-X vector (BD Clontech). The generated recombinant adenovirus plas-
mids were sequenced. The recombinant adenovi-
rus was prepared according to the manufac-
turer’s instructions and titrated using Adeno-X
Rapid Titer kits (BD Bioscience).

Preparation of DCs and CIKs

Using Ficoll-Paque, PBMCs were separated
from 70 mL peripheral blood and cultured in
RPMI-1640 supplemented with 10% (V/V)
autologous serum at 1×10⁶/mL. Informed con-
sent was obtained from the participants in
accordance with the study protocols approved
by the Institutional Review Board of Hubei
University of Arts and Science. Subsequently,
the obtained cells were transferred into culture
flasks and cultured for 2 hrs. Nonadherent cells
collected as the progenitor of CIKs were resus-
pended at 1×10⁶ cells/mL in RPMI-1640 medi-
um supplemented with 10% human AB serum
and cultured with immobilized anti-CD3 anti-
body (10 μg/mL, BD Pharmingen, NJ, USA) and
rhIL-2 (500 U/mL) as well as rhIFN-γ (1000 U/
ml) in culture flask. Half the amount of medium
was exchanged every three days and supple-
mented with rhIL-2 and rhIFN-γ. The remaining
adherent cells were cultured in RPMI-1640
medium (containing 40 ng/mL rhGM-CSF and
40 ng/mL rhIL-4). The supernatant was re-
placed with fresh medium containing rhGM-
CSF and rhIL-4 every 3 days. All cultures were
incubated at 37°C in 5% humidified CO₂.

Analysis of SOCS1 silencing by western blot

After 7 days of culture, the ex vivo cultured
DCs were washed and placed in 6-well plates
at 2.5×10⁵ cells/well with 600 μL RPMI-1640.
The cells were exposed to Ad-shSOCS1 or Ad-
shControl at 100 MOI. After 8 h transduction,
the cells were washed with PBS and further
incubated in fresh medium for 48 h, and then
collected and lysed with cell lysis buffer. The
protein samples separated via SDS-PAGE were
transferred onto immobilon membranes (Milli-
pore, MA, USA). SOCS1 and β-actin were identi-
fied by anti-SOCS1 polyclonal and anti-β-actin
monoclonal antibodies (Santa Cruz Biotechno-
logy, USA), respectively.

Maturation and cytokine analysis of DCs via
ELISA

After 7 days of culture as described in “Prepar-
ation of DCs and CIKs”. The DCs were washed
and placed in 12-well plates at a concentration
of 1×10⁵ cells/well in 400 μL RPMI-1640. The
DCs were exposed to Ad-shRNA-SOCS1 or ad-
shRNA-Control at 100 MOI. After 8 h transduc-
tion, the cells were washed with PBS and fur-
ther incubated in fresh culture medium. The
DCs were then pulsed with the HPVm16E7 pro-
tein at 25 μg/mL (HPVm16E7 was prepared as
we previously reported in [22]) for 6 h, followed
by stimulation with TNF-α (10 ng/mL) for 24 h
to develop mature DCs. IL-12p70 and IL-
6 contents (absorbance) in the supernatants
were quantitated separately by ELISA (Dakewe
Biotech Co. Ltd., China) according to the manu-
facturer’s instructions. Using FACS, the pheno-
type molecules of HLA-DR/CD1a/CD80/CD83/
CD86-fluorescein isothiocyanate (FITC) were
measured to investigate DC maturation (Santa
Cruz Co, Ltd.), three repeats for each group.

Phenotype detection of DCCIKs and secretion
levels of TNF-α and IFN-γ

CIKs cultured without rhIFN-γ were mixed with
HPVm16E7-pulsed DCs which had been trans-
ducted with Ad-shRNA-SOCS1 or Ad-shRNA-
Control at 100 MOI, stimulated by TNF-α, and
cultured for 8 days at a ratio of 10:1. The cell
mixtures were then cultured in CIKs medium
for 3 days. CIKs, Ad-shSOCS1-DCCIKs, and
Ad-shControl-DCCIKs were collected for the
detection of the CIKs surface markers. CD3,
CD3⁺CD56⁺, and CD3⁺CD8⁺ were examined by
FACS analysis. The effector cells cultivated
without rhIFN-γ was also cocultured with tar-
get SiHa cells constitutively expressing HPV16
E6 and E7 at effector/target (E/T) ratios of
10:1, 30:1, and 90:1 for 24 h. Levels of TNF-α
and IFN-γ in the supernatant of CIKs, Ad-
shSOCS1-DCCIKs, and Ad-shControl-DCCIKs at
10:1, 30:1 and 90:1 E/T ratios were tested by
ELISA (R&D Systems, Minneapolis, MN). The
TNF-α and IFN-γ ELISA assays were performed following the instruction manual.

In vitro antitumor activity analysis

The in vitro cytotoxicity of the CIKs, Ad-shSOCS1-DCCIKs, and Ad-shControl-DCCIKs against SiHa cells was determined using a CCK8 kit, (Dojindo Molecular Technologies, Inc., Kumamoto, Japan). The CIKs, Ad-shSOCS1-DCCIKs and Ad-shControl-DCCIKs harvested 11 days after induction were used as effector cells. The SiHa cells were used as target cells. The effector and target cells were added to 96-well plates at a ratio of 10:1, 30:1 and 90:1. The cells were incubated for 24 h. The groups comprising a mixture of cell types were the experimental groups, while the control groups contained only one cell type of the SiHa, CIKs, Ad-shSOCS1-DCCIKs, Ad-shControl-DCCIKs or 1640-RPMI cultivating solution. The CCK8 assay was performed in triplicate to evaluate cell viability, and optical density (OD) was read at 570 nm.

In vivo antitumor assay

Xenografted tumor mouse models were established by abdominal subcutaneous injection of 1×10⁶ SiHa cells into 6-week-old female BALB/c nude mice. On day 9, all mice had palpable tumors were assigned to 4 groups: PBS (served as control), CIKs, Ad-shSOCS1-DCCIKs, and Ad-shControl-DCCIKs (10 mice in each group). Therapeutic treatments were initiated with a total of 1.0×10⁷ CIKs, Ad-shSOCS1-DCCIKs, Ad-shControl-DCCIKs or PBS, respectively, 2.5×10⁶ cells (per dose) on the day of 9, 13, 17 and 21 were administered intravenously into the mice tail for a total of 4 injections. The tumor volume was calculated by the following formula: (major axis × minor axis²) ×0.52. When the tumor volume reached up to 2,500 mm³, the tumor-bearing mice were euthanized to avoid unnecessary pain.

In vivo metastasis assay by heterotopic liver implantation

To observe the reversed metastatic potential by HPVm16-E7-pulsed Ad-shSOCS1-DCCIKs on SiHa cells, 3×10⁶ SiHa cells were injected subcutaneously into nude mice. As the tumor cube implantation in the liver has advantages over injecting cells to ensure tumor formation in liver in order to observe heterotopic tumor metastasis from the liver to the lung, the subcutaneous formed tumors were then implanted as 1.2 mm³ cubes into the left liver lobes of nude mice. Post 9 days of implantation, Therapeutic treatments of PBS, CIKs, Ad-shSOCS1-DCCIKs, and Ad-shControl-DCCIKs with a total of 1.0×10⁷ cells were administered (10 mice in each group). A total of 4 consecutive injections every 5 days were administered with 2.5×10⁶ cells (per dose). After 6 weeks, the mice were sacrificed for lung metastasis assay examined by sectioning and microscopy. All groups were euthanized simultaneously at 6 weeks after tumor cube implantation.

Results

Morphological observation of DCs

After pulsed with HPVm16E7 and stimulated with TNF-α, DCs in irregular shape presented mature characteristics of larger soma with plenty of dendritic long and small bulges on their surfaces under scanning microscopy (Figure 1).

Inhibition of SOCS1 expression

 Constructs of Ad-shSOCS1 and Ad-shRNA-Control were verified in DCs (Figure 1). Western blot analysis demonstrated that Ad-shControl infection at MOI 100 greatly reduced SOCS1 expression compared with Ad-shControl.
SOCS1 silenced DCs improved therapy of CIKs

Table 1. Percentage of DCs expressing CD1a, CD80, CD83, CD86, HLA-DR

<table>
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<tr>
<th></th>
<th>CD1a</th>
<th>CD80</th>
<th>CD83</th>
<th>CD86</th>
<th>HLA-DR</th>
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<tr>
<td>Control</td>
<td>27.2±3.3</td>
<td>31.2±6.5</td>
<td>38.9±6.3</td>
<td>42.5±5.4</td>
<td>57.6±5.8</td>
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<td>TNF-α</td>
<td>82.4±5.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>72.4±8.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>79.2±8.2&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>90.3±5.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ad-shControl-DCs</td>
<td>83.1±7.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>76.1±7.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>81.2±5.2&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>Ad-shSOCS1-DCs</td>
<td>93.6±4.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>89.6±6.1&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>94.6±2.5&lt;sup&gt;d&lt;/sup&gt;</td>
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Immature DCs generated from PBMCs were stimulated by GM-CSF and IL-4 for 7 days and further stimulated by Ad-shSOCS1 or Ad-shControl. Percent of DCs expressing CD1a, CD80, CD83, CD86 and HLA-DR were detected using flow cytometry before and after 24 h TNF-α stimulation and analyzed by SPSS one-way ANOVA (Student’s t test) analysis. *Significant differences compared with that of the DCs control, P<0.001; <sup>a</sup>No significant differences compared with that of the TNF-α control, P>0.05; <sup>b</sup>Significant differences compared with that of the TNF-α control or Ad-shControl group, P<0.05; <sup>c</sup>Significant differences compared with that of the TNF-α control, P<0.05, but no significant differences compared with that of the Ad-shControl group, P>0.05.

Evaluation of DCs maturation

Immature DCs (iDCs) produced from PBMCs were stimulated with GM-CSF and IL-4 for 7 days, and further stimulated with Ad-shSOCS1 and HPVm16E7. Before and after 24 h of TNF-α stimulation, the expression levels of the surface markers CD1a, CD80, CD83, CD86 and HLA-DR were analyzed by flow cytometry and SPSS one-way ANOVA analysis. The results in Table 1 demonstrated that the expression levels of all antigens significantly increased by 2-4 folds during maturation in both Ad-shSOCS1 and Ad-shControl infected DCs with respect to control. The expression levels of CD1a, CD80 and CD83 increased about 10% in the former more than the latter. In addition, although TNF-α greatly induced the expression of these CD markers, it was lower than that of the Ad-shSOCS1 group in CD1a, CD80 and CD83 expression.

IL-12 and IL-6 analysis in DCs

Such proinflammatory cytokines as IL-12 (IL-12p70) and IL-6 induced by HPV16mE7-pulsed DCs with or without TNF-α stimulation were quantitated by ELISA analysis kits (Figure 2). Stimulated by TNF-α, the expression of IL-12p70 and IL-6 was considerably enhanced in all groups, while silencing SOCS1 in HPV16-
mE7-pulsed DCs induced far more cytokines than that of other groups.

**Cellular phenotype detection of DCCIWs and secretion levels of TNF-α and IFN-γ**

Both CD3+CD8+ and CD3+CD56+ double positive cells were significantly improved after co-cultivation with Ad-shControl-DCs or Ad-shSOCS1-DCs in CIKs culture medium. Further, the CD3+CD56+ population in the Ad-shSOCS1-DCs ICWs were improved about 42.5% compared to that of Ad-shControl-DCs (Table 2). The TNF-α and IFN-γ levels in the supernatant of group Ad-shSOCS1-DCs were highest, versus Ad-shControl-DCs and CIKs in CIKs culture medium without IFN-γ. And levels in group Ad-shControl-DCs were also higher than those of CIKs. It was demonstrated that after co-culture of CIKs and Ad-shSOCS1-DCs or Ad-shControl-DCs, IFN-γ and TNF-α levels in the supernatants were apparently increased (Figure 3).

**In vitro antitumor activity analysis**

Using CCK-8 assay, the cytotoxicity of effector cells at 10:1, 30:1 and 90:1 E/T ratios against SiHa cells was analyzed. It revealed that Ad-shSOCS1-DCs ICWs exhibited the highest cytotoxic activity against SiHa cells compared with Ad-shControl-DCs or CIKs. *P<0.01, analyzed by Student’s t-test.

**In vivo antitumor activity analysis**

The therapeutic effect of the Ad-shSOCS1-DCs lymphocytes against mice bearing tumors was investigated in BALB/C nude mice received 1×10⁶ SiHa cells. When all mice had palpable tumors on day 9, the therapeutic treatments were initiated with different T cells.
SOCS1 silenced DCs improved therapy of CIKs

or PBS. As shown in Figure 5A, the mice treated with Ad-shSOCS1-DCCIKs exhibited the greatest survival days of 60 (40%) compared with the mice treated with Ad-shControl-DCCIKs (0%), CIKs (0%) and PBS (0%). Tumor volume experiment demonstrated that Ad-shSOCS1-DCCIKs lymphocytes significantly regressed the tumor volume compared with CIKs and PBS controls in which progressive tumor growth was observed. The experiments were repeated, producing a significant statistical difference (P< 0.01) (Figure 5B). Although therapeutic Ad-shSOCS1-DCCIKs could not always eradicate the tumor completely, the survival time was significantly prolonged more than 60 days in 40% of the Ad-shSOCS1-DCCIKs treated mice in respect to the mice in PBS control group which were all dead in 40 days. These results indicated the therapeutic potential of Ad-shSOCS1-DCCIKs against HPV16 E7 tumor in vivo.

In vivo metastasis assay

To observe the antimetastatic potential of HPVm16E7-pulsed Ad-shSOCS1-DCCIKs on SiHa cells, the subcutaneous formed SiHa tumor cubes at 1-2 mm³ were implanted into the left liver lobes of nude mice, as the tumor cube implantation in the liver has advantages over injecting cells to ensure tumor formation in liver. Figure 6B showed normal lung tissue against Figure 6A in which a representative metastatic lesion was demonstrated. As demonstrated in Figure 6C, Ad-shSOCS1-DCCIKs and Ad-shControl-DCCIKs lymphocytes decreased the tumor metastasis from the liver to the lung in the heterotopic liver implantation models (20%), whereas a high metastasis rate (70%) was observed in the PBS control group.

Discussion

Stimulated with IFN-γ, IL-2, and CD3 monoclonal antibody (OKT3), CIKs expressing the CD3/CD56 surface markers of T and NK cells are a cell population obtained from peripheral blood mononuclear cells [16]. Possessing the strongest tumor cytotoxicity with an extensive range of tumor killing, CIKs can both directly suppress tumor cells and regulate the body immune system against tumor cells indirectly. Therefore, CIKs can inhibit the tumor’s growth and recurrence by immediate cytotoxicity on tumor cells and improve the patients’ immunity for long-term effect.

As the antigen-presenting DCs were able to induce robust cell-mediated immunity capable of attacking and eliminating abnormal antigen-bearing cells, allergization for CIKs with tumor antigen-pulsed DCs can both exert non-MHC restrictive cytotoxicity of CIKs and acti-
SOCS1 silenced DCs improved therapy of CIKs

In vivo metastasis assay by heterotopic liver implantation: a subcutaneously formed tumor in 1-2 mm³ cubes generated from SiHa cells was implanted into the left liver lobes in nude mice. After 9 days of tumor cube implantation, therapeutic treatments of PBS, CIKs, Ad-shSOCS1-DCCIKs, and Ad-shControl-DCCIKs with a total of 1.0×10⁷ cells were administered intravenously into the tail of the mice, PBS served as control (10 mice in each group). A total of 4 consecutive injections every 5 days were administered with 2.5×10⁶ cells (per dose). After 6 weeks, the mice were sacrificed for lung metastasis assay examined by sectioning and microscopy. A. Representative tumor lesion in the lung, indicated by an arrow. B. No tumor lesion in the lung. C. Reversion of lung metastasis. Ad-shSOCS1-DCCIKs and Ad-shControl-DCCIKs lymphocytes decreased the tumor metastasis from the liver to the lung in the heterotopic liver implantation model (20% and 40%, respectively), whereas a high metastasis rate (70%) was observed in the PBS control group. *P<0.01, analyzed by Student’s t-test.

Figure 6.

The capacity of DCs to capture, process and present antigens varies in accordance with their differentiation/maturation stage and origin. In contrast to activated mature DCs which can induce protective anti-tumor immunity, immature DCs present self-antigens to T cells leading to immune tolerance by generating suppressor T cells or T cell deletion [14-16]. As activation of the full immunostimulatory talent of DCs to achieve an effective immunity shows potential against tumor, SOCS1, a negative signaling regulator of various cytokines was knocked down in DCs in this study. And the efficiency of SOCS1 silencing was verified. At MOI 100, SOCS1 was significantly knocked down in DCs (Figure 1B). The expression levels of the surface markers CD1a, CD80, CD83, CD86, and HLA-DR analyzed by flow cytometry were increased by 2-4 folds during maturation in both Ad-shSOCS1 and Ad-shControl infected and HPV16mE7 pulsed DCs with respect to control, but not in the absence of TNF-α stimulation (Table 1). Furthermore, the former increased about 10% more than the latter, indicating an increased maturation by silencing SOCS1. In addition, although TNF-α greatly induced the expression of these CD markers, it was lower than that of the Ad-shSOCS1, which implies that SOCS1 knock down enhances the effect of TNF-α on stimulating DCs maturation. This might be attributed to the increased NF-κB expression by silencing SOCS1, as well as promoting the efficacy of TNF-α on up-regulating NF-κB [23, 24]. SOCS1-restricted DCs also significantly enhanced the secretion of pro-inflammatory cytokines such as IL-12 and IL-6 (Figure 2).

The co-culture of CIKs with DCs can increase their proliferation activity and cytotoxicity [20]. As demonstrated in Figure 3, TNF-α and IFN-γ in the supernatants of the co-cultured Ad-shSOCS1-DCCIKs and Ad-shControl-DCCIKs were higher than those of CIKs. The CD3⁺CD8⁺ and CD3⁺CD56⁺ double positive cells in Ad-shSOCS1-DCCIKs and Ad-shControl-DCCIKs population were significantly higher than those of the CIKs group. Furthermore, the CD3⁺CD56⁺ double positive cells in the former were improved 42.5% than the latter, which indicates silencing SOCS1 improved the efficacy of DCs on irritating proliferation of CIKs. Moreover, the cytotoxicity of effector cells at 90:1, 30:1 and 10:1 E:T ratios revealed that Ad-shSOCS1-DCCIKs lymphocytes exhibited the highest cytotoxic activity against SiHa cells by CCK-8 assay. The cytotoxic activity against SiHa cells induced by Ad-shSOCS1-DCCIKs and Ad-shControl-DCCIKs was not linear, but it increased with E:T ratio compared to the linear increase of the control group, indicating a dosage-
dependent manner. The strengthened cytotoxic activity on tumor of DCCIKs may be associated with the ascending secretion of IFN-γ and IL-12 as well as increased CD3+CD56+ double positive cells in DCCIKs. And the increased IFN-γ and IL-12 in co-cultured DCCIKs further stimulated the CIK’s proliferation [12-13].

To further investigate the antitumor effects mediated by the Ad-shSOCS1-DCCIKs, BALB/c-nude mice models xenografted with SiHa cells were developed. The tumor-loaded mice received injection of Ad-shSOCS1-DCCIKs and control T cells (2.5×10⁶ per dose) for a total of 1×10⁷. Tumor volume curve demonstrated that Ad-shSOCS1-DCCIKs lymphocytes significantly decreased the tumor volume compared with CIKs and PBS controls in which progressive tumor growth was observed. Statistical analysis of the tumor growth curves revealed significant differences between the Ad-shSOCS1-DCCIKs group and the control group (P<0.05). Although therapeutic Ad-shSOCS1-DCCIKs could not always eradicate the tumor completely, the survival rate was significantly enhanced (40%) at day of 60 compared with the mice treated with Ad-shControl-DCCIKs (0%), CIKs (0%) or PBS (0%). These results indicated the anti-HPV16 E7 tumor therapeutic potential of the HPV16mE7 pulsed and SOCS1 silenced Ad-shSOCS1-DCCIKs in vivo.

As the cure rate in patients with high-grade cervical cancer remains very low in part by the high recurrence rate, the antimetastatic potential of Ad-shSOCS1-DCCIKs on SiHa cells was assessed via heterotopic tumor metastasis assay from the liver to the lung. The result demonstrated Ad-shSOCS1-DCCIKs efficiently inhibited liver tumor metastasis in vivo in the mouse models.

In conclusion, compared with Ad-shControl-DCCIKs or CIKs, Ad-shSOCS1-DCCIKs showed prominent oncotherapy effect and powerful anti-metastatic efficacy, presenting a favorable application prospect.

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Disclosure of conflict of interest

None.

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