RESEARCH COMMUNICATION

Enhanced Induction of Anti-tumor CTLs in Vitro by a Lentivirus-transduced dendritic Cell Vaccine Expressing Secondary Lymphoid Tissue Chemokine and Mucin 1

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Abstract

Aims: Dendritic cell (DC)-based cancer immunotherapy requires an immunogenic tumor associated antigen (TAA) and an effective strategy for its presentation to lymphocytes. Here, we explored whether transduction of DCs with lentiviruses (LVs) expressing a fusion protein of secondary lymphoid tissue chemokine (SLC) and mucin 1 (MUC1) could stimulate antigen-specific cytotoxic T cells (CTLs) to human cancer cells in vitro. Materials and methods: HLA-A2+ peripheral blood monocyte-derived DCs were transduced with recombinant lentiviruses at different multiplicities of infection (MOI), and MUC1, SLC or SLC-MUC1 mRNA and protein were detected by RT-PCR and Western blotting, respectively. Transduction efficiencies and phenotypes of DCs were evaluated by flow cytometry. Induction of T lymphocyte proliferation by DCs was examined with a Cell Count Kit-8 (CCK-8). CTL activities against tumor cells were analyzed by lactate dehydrogenase (LDH) cytotoxicity and enzyme-linked immunospot (ELISPOT) assays. Results: Stable expression of MUC1, SLC and SLC-MUC1 was obtained in DCs transduced with recombinant LVs, and the transduction efficiencies were dose-dependent. Transduction with LVs did not appreciably change the DC phenotype. CTL induced by LV MUC1 DCs potently and specifically lysed the HLA-A2+, MUC1+ colon cancer cell line HCT-116. Moreover, this cytolytic activity against HCT-116 was enhanced with CTL stimulated by LV SLC-MUC1 DCs. Conclusions: DCs transduced with MUC1 could induce effective cytolytic activity against tumor cells in an antigen-specific and HLA-restricted fashion in vitro, and SLC promoted MUC1-specific anti-tumor activity. The transduction of DCs with LV SLC-MUC1 may be a promising strategy in DC-based cancer immunotherapy.

Keywords: Dendritic cell - MUC1 - SLC - CTL - immunotherapy

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Introduction

Mucin 1 (MUC1) is a type I transmembrance protein that consists of an extracellular domain containing a variable number of tandem repeats (VNTRs), a transmembrance domain and a cytoplasmic domain (Gendler., 2001; Schuman et al., 2005). The 20-amino acid sequence (GVTSAPDTRPAPGSTAPPAH) repeats of the VNTRs of MUC1 are heavily glycosylated (Gendler et al., 1995). While MUC1 is expressed at low levels on the apical surface of ductal epithelia cells in many organs, on tumor cells it is overexpressed, under-glycosylated and associated with loss of polarity (Croce et al., 2007; Seregni et al., 1997; Brockhausen et al., 1995). These characteristic changes of MUC1 exposes its protein backbone, thereby making the tumor vulnerable to the immune system. Therefore, MUC1 epitopes are considered potential anti-tumor targets. Previous studies have attempted to develop MUC1 for cancer immunotherapy, mostly focusing on the tandem repeats in the extracellular domain, which form the most immunogenic part of the protein (Gilewski et al., 2000; Soares et al., 2001).

An effective cancer vaccine relies not only on a suitable tumor associated antigen (TAA) but also on a strategy that effectively presents it to the immune system. Dendritic cells (DCs) are potent antigen-presenting cells capable of regulating both innate and adaptive immune responses (Palucka et al., 1999; Banchereau et al., 2000). Thus, one of the most promising methods being developed in cancer immunotherapy is the use of TAA-loaded dendritic cells (DCs) as powerful antigen-presenting carriers. Both cellular and humoral immune systems are involved in generating anti-tumor activities, and DCs play crucial roles in eliciting these responses. TAAs are mainly presented by DCs through the MHC class I pathway which stimulates cytotoxic T lymphocytes (CTLs) that consequently kill

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tumor cells bearing those epitopes. Meanwhile, a relatively smaller number of TAA epitopes are presented through the MHC class II pathway and recognized by CD4+ T cells (Topalian et al., 1996; Renkvist et al., 2001; Brossart et al., 1997). Immune responses primed and expanded by DCs are regulated by signals acting through various soluble mediators (i.e., cytokines and chemokines) (Ferrantini et al., 2008). Previous studies have demonstrated that when combined with TAA, certain cytokines can enhance vaccine-induced anti-tumor effects (Sikora et al., 2009; Steel et al., 2010).

Secondary lymphoid tissue chemokine (SLC), a CC chemokine, is expressed in high endothelial venules and in T cell zones of spleen and lymph nodes. SLC has a strong ability to attract naïve T cells and DCs to the secondary lymphoid organs and to activate T cells (Hromas et al., 1997). The anti-tumor effect of SLC has been well demonstrated in murine models. However, little research has been done to investigate the potential anti-tumor effect of the combination of human SLC and TAAs. In this study, we investigated whether DCs transduced with a lentiviral vector could elicit effective MUC1-specific anti-tumor CTL responses in vitro and evaluated the potency of SLC as an adjuvant to boost these activities.

Materials and Methods

Reagents and cell culture

Recombinant human interleukin-4 (rhIL-4) and recombinant human granulocyte-macrophage colony-stimulation factor (rhGM-CSF) were purchased from R&D systems (Minneapolis, MN). Lipopolysaccharide (LPS) from Escherichia coli was purchased from Sigma-Aldrich (Saint Louis, MO). Monoclonal antibodies specific for CD1a, CD11c, CD14, CD80, CD83, CD86, human leukocyte antigen DR (HLA-DR) and their isotype-control antibodies were purchased from BD Pharmingen (San Diego, CA). Cell Count Kit-8 (CCK-8), CytoTox96 cytotoxicity detection kit and ELISPOT kit were purchased from Dojindo (Kumamoto, Japan), Promega (Madison, WI) and Dakewei (Shenzhen, China), respectively.

The colonic cancer cell lines HCT-116 (MUC1+, HLA-A2+), LOVO (MUC1-, HLA-A2-) and the gastric cancer cell lines MKN-28 (MUC1-, HLA-A2+) and SGC-7901 (MUC1+, HLA-A2-) were maintained in our laboratory. All these cell lines were cultured in DMEM supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin-streptomycin and 2 mM L-glutamine.

Construction of recombinant lentiviral vector

The gene fragment encoding five tandem repeats of MUC1 and SLC-MUC1 fusion gene were synthesized commercially (Takara, Dalian, China). After amplification by PCR, gene fragments of MUC1, SLC and SLC-MUC1 were purified, digested and inserted into the lentiviral (LV) expression vector pLV-GFP (kindly provided by Yun Gao, Liver Transplantation Center of the First Affiliated Hospital and Cancer Center, Nanjing Medical University, Nanjing, Jiangsu Province, PR China). Construction of the recombinant LV vector was performed as previously described (Gao et al., 2010). Subsequently, the integrities of the three recombinant lentiviral vectors were confirmed by restriction digestion and sequencing.

Production of LVs

LVs were produced in 293T cells by co-transfection of three plasmids, the recombinant vector plasmid, packaging plasmid pCMVΔ8.91 and envelope plasmid pLR/VSV-G by calcium phosphate as previously described (Dull et al., 1998). Supernatants were collected 48 and 72 h after transfection, and the LVs were concentrated by ultracentrifugation(50,000g, 2 hours, 4°C).The viral pellets were resuspended in RPMI 1640 and stored at -80°C. The titers of the concentrated viruses were determined by infecting 293T cells with serial dilutions of the virus stock in the presence of polybrene in 6-well plates.

Generation and LV transduction of DCs

Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll-Hypaque density gradient centrifugation from buffy coats of healthy HLA-A2+ donors. CD14+ cells were positively selected from the PBMCs by microbead labeling and magnetic cell separation (MACS, Miltenyi Biotec) according to the manufacturer's instructions. The purity of the CD14+ monocytes was typically >95% determined by flow cytometry. The CD14+ monocytes were cultured in RPMI-1640 medium containing 10% FBS, rhGM-CSF (1000 U/ml) and rhIL-4 (500 U/ml) for 5 days. LPS (1 μg/ml) was used to induce maturation of DCs at day 5 for an additional 2 days. For LV transduction, DCs were infected on day 3 at a multiplicity of infection (MOI) of 5–20 in 6-well plates with 1×10^6 DCs per well in the presence of 8 μg/ml polybrene. After 12 h the DCs were washed with phosphate-buffered saline (PBS) and resuspended in fresh RPMI-1640 containing 10% FBS, rhGM-CSF and rhIL-4. LPS was added on day 5, and the cell morphology was observed under a microscope. The transduction efficiency of DCs was also determined by fluorescence microscopy and further analyzed by flow cytometry.

Flow cytometric analysis

The cell surface phenotypes of DCs on day 5 (immature DCs), DCs on day 7 (mature DCs) and infected DCs on day 7 were analyzed using fluorescein isothiocyanate (FITC)-, phycoerythrin (PE)- or PE-carbocyanin (Cy)5-labeled monoclonal antibodies against CD1a, CD11c, CD14, CD80, CD83, CD86, HLA-DR or an isotype control. DCs were collected, washed and resuspended in PBS, then incubated with antibodies for 30 min on ice. After washing, cells were analyzed by flow cytometry.

Expression of MUC1, SLC and SLC-MUC1 in LV-infected DCs

Expressions of MUC1, SLC and SLC-MUC1 in LV-infected DCs were detected at both the transcript and protein levels. After transduction for 96 h, DCs were collected on day 7, and total RNA and protein were prepared for detection by RT-PCR and Western Blot, respectively. LV-GFP-infected DCs and uninfected DCs

were evaluated in parallel as controls. PCR primers for MUC1 were F:5'-ACTCGACCTGCACCTGGATC-3',R:5'-GGCCTAGTATCTGGCGCACT-3' and F: 5'-GCCTCCTTATCCTGGTTCTG-3', R:5'-TGGGCTGGTTTCTGTGGG-3' for SLC, primers for SLC-MUC1 were F: 5'-GCCTCCTTATCCTGGTTCTG-3'and R:5'-GGCCTAGTATCTGGCGCACT-3'. A mouse monoclonal antibody of V5-Probe (sv5-pk) and a rabbit polyclonal antibody to SLC (Santa Cruz Biotech, Santa Cruz, CA) were used in Western Blot analysis of the LV transduced DCs. GAPDH was detected as a loading control.

Mixed lymphocyte reaction (MLR) assay

PBMCs were seeded in 6-well plates and incubated for 2 h in 5% CO_2 at 37°C to allow plastic adherence of the monocytes. Non-adherent cells were then collected as peripheral blood lymphocytes (PBL). T cells for the MLR assay were purified using a Nylon Fiber Column T (Wako, Japan) according to the manufacturer's instructions. Briefly, after washing and equilibration of the Nylon Fiber Column, PBLs suspended in DMEM (5% FBS) at 4°C were added to the column. After adding 1 ml DMEM (5% FBS) at 37°C to the column, the top of the column was covered with aluminium foil and incubated at 37°C for 45–60 min. After incubation, purified T cells were obtained by collecting the cell suspension eluted from the column. DCs were pre-treated with mitomycin C (MMC) and then co-cultured in 96-well round-bottom culture plates with T cells (2×10^5/well) at a ratio of 1:10 to 1:100 for 96 h at 37°C. CCK-8 solution was added into each well and incubated for 4 h before measuring OD values in a microplate reader at 450 nm.

Cytotoxicity assay

Autologous CD8+T cells were enriched from PBMCs of HLA-A2+ donors using a Dynal CD8 positive Isolation Kit (Invitrogen, USA) according to the manufacturer's instructions. CTLs were generated in vitro by weekly stimulation of CD8+T cells with MMC pre-treated DCs at a ratio of 10:1. After three cycles of stimulation, cells collected on the 21st day were used as CTLs. CTL activity was evaluated using a non-radioactive cytotoxicity detection kit based on lactate dehydrogenase (LDH) release. After washing, the target cells were counted and seeded in 96-well V-bottomed culture plates at 1×104 per well. Varying numbers of CTLs were added to a final volume of 100 μl at the effctor to target (E/T) ratios of 5:1, 10:1 and 20:1 and incubated for 4 h at 37°C. The supernatants were harvested for the LDH assay and OD measurement in a microplate reader at 490 nm. The percent of specific lysis is defined by the formula: $(\text{Experimental} - \text{Effector}_{\text{Spontaneous}} - \text{Target}_{\text{Spontaneous}})/(\text{Target}_{\text{Maximum}} - \text{Target}_{\text{Spontaneous}}) \times 100\%$.

Enzyme-linked immunospot (ELISPOT) assay

The ELISPOT assay was performed to determine the frequency of IFN-γ-producing CTLs stimulated by target tumor cells using 96-well culture plates pre-coated with anti-human IFN-γ (Dakewei) according to the manufacturer's instructions. CTLs were co-cultured with the target HCT-116 cells (1×10^4 per well) at the E/T ratio of 20:1. After incubation for 24 h at 37°C without moving the plate, cells were removed and plates washed. Biotinylated IFN-γ mAb (1 h at 37°C) was added, followed by streptavidin-HRP (1 h at 37°C) and then pre-mixed AEC solutions with washes between each step. The reaction was stopped with distilled water upon development of the spots, which were subsequently quantified with an ELISPOT reader.

Results

Characterization and LV transduction of CD14+ derived DCs

To characterize the DCs isolated from CD14+ monocytes, the cells were cultured with GM-CSF plus IL-4 or stimulated by LPS and observed by light microscopy. The DCs showed typical morphologies. The immature DCs (day 5, Figure 1a) were observed to be more rounded, while the mature DCs (day 7, Figure 1b) were adherent with typical dendritic processes.

LVs were produced by co-transfection of 293T cells with the triple plasmid system, and the titers of the concentrated viruses were determined by infecting 293T cells with serial dilutions as described in Materials and Methods. The titers of the virus stocks were calculated to be 5×10^7–1×10^8 transduction units (TU)/ml based on the formula: (% GFP+ cells) × (target cell numbers) × (1/dilution).

To optimize the transduction efficiency in DCs, the cells were infected with LV on day 3 at different MOI (5–20). After 96 h of infection, high levels of GFP expression in DCs were observed under a fluorescence microscope, and the transduction efficiency determined by flow cytometry was shown to increase in an dose-dependent manner (Figure 1). At 5 MOI, the transfection efficiency was only 14.91% and increased to 25.65% at

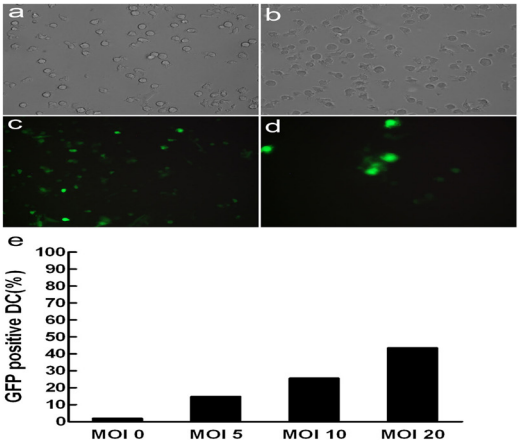

Figure 1. Morphology of Immature DCs and Mature DCs. a) DCs were cultured with GM-CSF plus IL-4 and collected on day 5 as immature DCs. b) Mature DCs were obtained by LPS stimulation on day 5 for two days. Typical morphologies of DCs were observed under a light microscope (400×). c, d) Lentiviral transduction of CD14+ derived DCs. Transduction of DCs was performed on day 3 (MOI=20), and the transduction efficiency was observed and detected after 96 h. GFP expression in DCs (200× and 400×). e)Transduction efficiency of DCs at various MOI

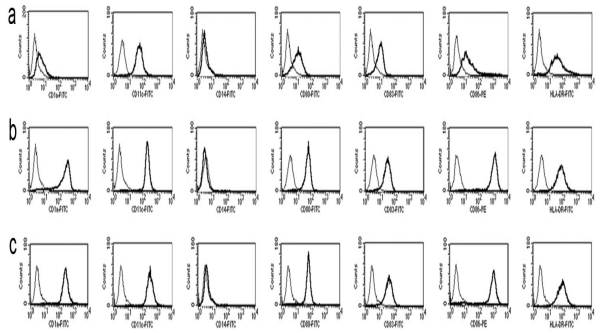

Figure 2. Phenotypic Analysis of Immature DCs, Mature DCs and LV-infected DCs by Flow Cytometry. Cell surface makers of CD1a, CD11c, CD14, CD80, CD83, CD86 and HLA-DR were detected in immature DCs (a), mature DCs (b) and LV-infected DCs (c). The results showed increased expressions of CD1a, CD11c, CD80, CD83, CD86, HLA-DR and decreased expression of CD14 in mature DCs compared with immature DCs. A representative experiment of three is shown

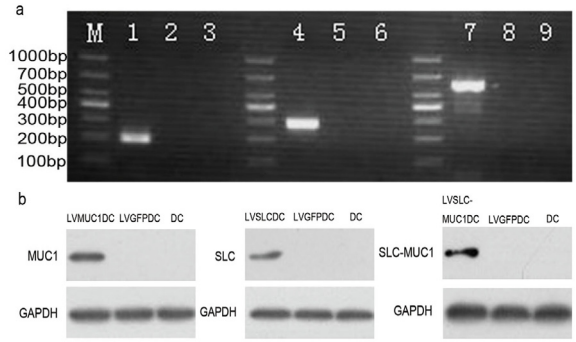

Figure 3. Expressions of MUC1, SLC and SLC-MUC1 in LV-infected DCs (MOI = 20). RT-PCR and Western Blot were used to detect the expressions of MUC1, SLC or SLC-MUC1 in infected DCs. LV-GFP DCs and DCs were controls. a, mRNA expressions of MUC1 (lane 1, 188 bp), SLC (lane 4, 280 bp) and SLC-MUC1 (lane 7, 589 bp). Lanes 2, 5, 8: LV-GFP DCs; lanes 3, 6, 9: DCs. b, Western blot analysis confirmed the protein expressions of MUC1, SLC and SLC-MUC1 in LV-infected DCs. Neither LV-GFP DCs nor uninfected DCs showed specific bands

10 MOI, with the highest transfection efficiency reaching 43.53% at 20 MOI.

Phenotypic characteristics of DCs

Flow cytometric analysis was used to detect whether LV transduction would affect the phenotype of DCs. Immature DCs (day 5), mature DCs (day 7) and LV-infected DCs (day 7) were collected for detection of cell surface markers. As expected, mature DCs showed upregulation of CD1a, CD11c, CD80, CD83, CD86, HLA-DR and downregulation of CD14 on the cell surface compared with immature DCs. Moreover, no significant difference in expression of the cell surface markers was observed between mature DCs and LV-infected DCs, indicating that the LV transduction did not alter the surface phenotype of DCs (Figure 2).

MUC1, SLC and SLC-MUC1 expression in LV transduced DCs

RT-PCR and Western Blot were carried out to detect the expressions of MUC1, SLC and SLC-MUC1 in DCs. Specific PCR bands for MUC1, SLC and SLC-MUC1

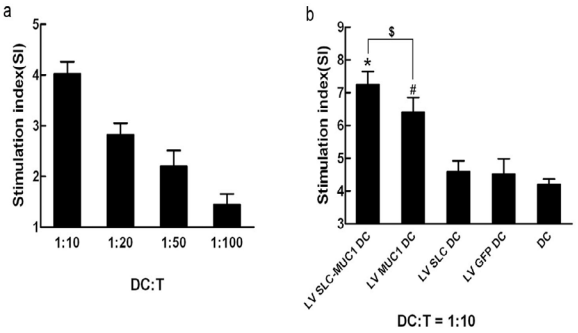

Figure 4. Proliferation of Allogeneic T Lymphocyte Stimulated with DCs in MLR Assays. DCs were co-cultured with 2×10^5 T cells in 96-well culture plates at various S/R ratios (1:10, 1:20, 1:50 or
1:100) for 96 h, and T lymphocyte proliferation was measured by a CCK-8 assay. a, Stimulation indices (SI) of uninfected DCs increased with higher stimulator numbers. b, At the S/R ratio of 1:10, SI of LV SLC-MUC1 DC or LV MUC1 DC group compared to the LV SLC DC, LV GFP DC or DC group was statistically significantly different (*, # $P < 0.01$); SI of LV SLC-MUC1 DC was higher than that of the LV MUC1 DC

(Figure 3a, lanes 1, 4, 7, respectively), while no bands were shown in control samples. Likewise, MUC1, SLC and SLC-MUC1 proteins were specifically detected by Western blot (antibody to MUC1 and SLC-MUC1 was sv5-pk) in the DCs transduced with LVs expressing the respective proteins and not in the LV-GFP transduced or non-transduced DCs (Figure 3b).

T cell proliferation

To access the ability of mature DCs to induce lymphocyte effector responses, CCK-8 was utilized to examine the allogeneic T lymphocyte proliferation in MLR assays. With increasing stimulator to responder (S/R) ratios, uninfected DCs showed a gradual increase in the ability to stimulate lymphocyte proliferation, as shown in Figure 4a. Furthermore, at a 1:10 S/R ratio, both the LV MUC1 DC and LV SLC-MUC1 DC groups displayed strong stimulatory capacities, and the stimulation indices (SI) of the two groups were statistically significantly different ($P < 0.01$) compared with LV SLC DC, LV GFP DC or DC group. The LV SLC-MUC1 DC group also showed a higher SI than the LV MUC1 DC group ($P < 0.05$, Figure 4b).

Cytotoxic effects

In the LDH cytotoxic analysis, HCT-116, SGC-7901, MKN-28 and LOVO were used as target cells at various E/T ratios (5:1, 10:1 or 20:1) to evaluate the specific cytotoxic activity. The results demonstrated that the cytotoxic activity against HCT-116 tumor cells (MUC1+, HLA-A2+) was considerably stronger in both the LV SLC-MUC1 DC and LV MUC1 DC groups compared to the other three groups (LV SLC DC, LV GFP DC and DC) at all E/T ratios ($P < 0.01$, Figure 5a). Meanwhile, the cytotoxic activity in the LV SLC-MUC1 DC group was higher than that in the LV MUC1 DC group at different E/T ratios ($P < 0.05$, Figure 5a). Furthermore, in contrast to the strong specific CTL activities induced by LV SLC-MUC1 DC and LV MUC1 DC against HCT-116 cells, the

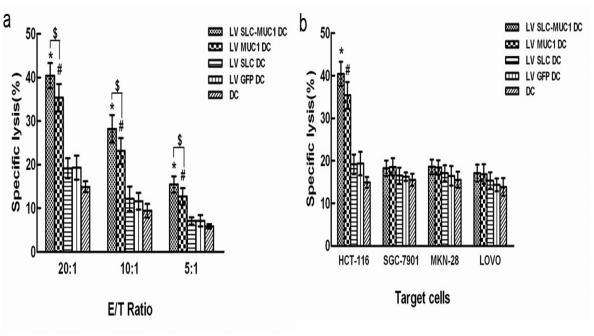

Figure 5. Detection of Cytotoxic activity of CTLs by LDH Assay. Autologous CD8+T cells were stimulated with LV-infected or uninfected DCs to generate CTLs. HCT-116, SGC-7901, MKN-28 and LOVO cells were used as targets. a, Specific CTL responses against HCT-116 were tested at different E/R ratios (20:1, 10:1 and 5:1). The percentages of cell lysis in the LV SLC-MUC1 DC group (40.42 ± 2.87%, 28.23 ± 3.16% and 15.48 ± 1.85%) were respectively higher than those of the LV MUC1 DC group (35.38 ± 3.15%, 23.14 ± 2.97% and 12.74 ± 1.91%) ($ P < 0.05). CTLs of both the LV SLC-MUC1 DC and LV MUC1 DC groups showed significantly greater capacities to lyse HCT-116 tumor cells than that of the LV SLC DC, LV GFP DC and DC groups (*,# P < 0.01). b, Antigen-specific CTL responses in vitro were evaluated by measuring cytolytic activities of CTLs targeting HCT-116, SGC-7901, MKN-28 and LOVO cells at the E/R ratio of 20:1. The results showed a significantly higher percentages of cell lysis of HCT-116 by CTLs in the LV SLC-MUC1 DC group (40.42 ± 2.87%) and LV MUC1 DC group (35.38 ± 3.15%) than those of the SGC-7901 (18.28 ± 1.81%, 18.57 ± 2.05%), MKN-28 (18.61 ± 1.70%, 18.46 ± 1.67%) or LOVO (17.12 ± 1.98%, 16.90±2.29%) cells (*, # P < 0.01). No significant difference between cytotoxic activity targeting SGC-7901, MKN-28 and LOVO cells in all DC groups was seen (P > 0.05)

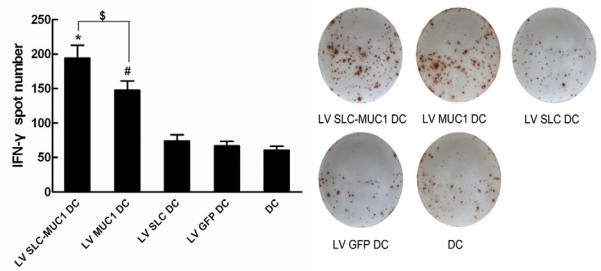

Figure 6. IFN-γ-secreting CTLs Determined by ELISPOT. CTLs induced by LV SLC-MUC1 DC, LV MUC1 DC, LV SLC DC, LV GFP DC or DC were co-cultured with 1 × 104 HCT-116 cells at E/T ratios of 20:1. IFN-γ secretion from CTLs of the LV SLC-MUC1 DC or LV MUC1 DC group was more than 2-fold higher than those of the LV SLC DC, LV GFP DC or DC group (*, # P < 0.01). Compared with those of the LV MUC1 DC group, the CTLs in the LV SLC-MUC1 DC group showed a significantly greater frequency of IFN-γ secretion ($ P < 0.01)

cytotoxic activities (E/T ratio of 20:1) detected against the SGC-7901 (MUC1+, HLA-A2-), MKN-28 (MUC1-, HLA-A2+) or LOVO (MUC1-, HLA-A2-) cells in all of the LV-transduced DC groups were not significantly different from that of the background level of the non-transduced DC group (Figure 5b).

IFN-γ secretion

ELISPOTS assays were used to detect IFN-γ secretion, representing specific activation, by CTLs induced by DCs with or without transduction of LV SLC-MUC1, LV MUC1 or LV SLC in the presence of tumor target cells. The cytotoxicity analysis above expectedly showed that the MUC1-specific cytotoxic effect could only be elicited by the MUC1+, HLA-A2+ HCT-116 tumor cells. Thus, detection of the frequency of IFN-γ-secreting CTLs was performed in the different groups with HCT-116 as the target cells, at the E/T ratio of 20:1. As shown in Figure 6, CTLs induced by LV SLC-MUC1 DC or LV MUC1 DC showed a significantly higher level of IFN-γ (>2-fold) than the three other groups (P < 0.01), with CTLs induced by LV SLC-MUC1 DC exhibiting the highest amount of IFN-γ secretion (P < 0.01, compared with LV MUC1 DC).

Discussion

DC-based anti-tumor immunotherapy has been a research focus in the past years, not only because of its powerful antigen-presenting function, but also because of its unique ability to stimulate naïve T lymphocytes and induce effective anti-tumor responses (Guermonprez et al., 2002). Since the first clinical trial for lymphoma (Hsu et al., 1996), DC-based vaccines have now been evaluated in clinical research for a variety of cancers, including melanoma, renal carcinoma and prostate cancer and achieved some promising results (Ranieri et al., 2000; Kugler et al., 2000; Small et al., 2000). An effective DC-based immunotherapy should contain a defined tumor antigen and a strategy to properly delivery it to DCs. As one of the few well-characterized tumor antigens (Vlad et al., 2004), human MUC1 is widely and abnormally highly expressed on various tumor cells, making it a promising and attractive TAA in anti-tumor immunotherapy. Vaccines based on MUC1 have demonstrated its effectiveness and functionality both in animal and human studies (Rong et al., 2009; Karanikas et al., 2001), and some have already entered phase III clinical trials (Apostolopoulos et al., 2006).

In our study, the synthetic MUC1 gene encoding five tandem repeats was used as the tumor antigen, and its potency in eliciting effective anti-tumor immune responses including tumor cell lysis was evaluated in vitro. Vaccination with MUC1-loaded DCs is becoming a popular strategy in immunotherapy for MUC1-positive tumors. However, the most effective and practical method for loading DCs with MUC1 has been under debate in past years. Gene-transduction of MUC1 to DCs is a feasible approach and can be performed using various strategies including electroporation, liposomes or recombinant virus (Pecher et al., 2001; Wang et al., 2009). LV vectors were chosen to transduce MUC1 into ex vivo-cultured DCs in our study for their powerful ability to deliver genes into non-dividing and terminally differentiated cells. Compared with other viral vectors, LVs have several obvious advantages in gene transduction, including high transduction efficiency, stable and long-term infection, minimal cytotoxicity and lack of induction of virus-specific immune responses (Kordower et al., 2000; Lizee et al., 2004). Although adenoviral vectors (AdV) have also been reported to be capable of transducing DCs efficiently,

they are highly immunogenic and thus may affect the anti-tumor immune responses (Molnar-Kimber et al., 1998). A recent study by TC Felizardo et al. (2011) demonstrated similarly high AdV- and LV-mediated antigen expression levels in DCs, but AdV-specific responses were observed after immunization with AdV-DCs.

In our study, we first determined the optimal dose for LV transduction by infecting DCs on culture day 3 at various MOI between 5–20. A satisfactory transduction efficiency of more than 40% was acquired at the MOI of 20, similar to that in a recently published report (Felizardo et al., 2011). The impact of LV infection on the maturation of DCs was further analyzed by profiling cell surface markers on immature DCs, mature DCs or LV-infected DCs. No apparent differences in the phenotypes were observed between mature DCs and LV-infected DCs. Both the effective presentation of TAA by mDCs and their recognition by lymphocyte effectors play crucial roles in anti-tumor immunity. Although, a MUC1-loaded DC vaccine had shown some encouraging results, it was proven to be inadequate and did not effectively reduce tumors (Wierecky et al., 2006). This result was mainly due to insufficient numbers of DCs migrating to the T cell zones after injection of the DC vaccine. Thus chemotactic factors to aid in recruitment of DCs to lymphocytes may be used to augment anti-tumor immunotherapy. SLC, also known as CCL21 or 6Ckine, is a potent chemokine for DCs and naïve T cells, known for its role in the co-localization of these two cell types and activation of cognate T cells (Chan et al., 1999). Turnquist et al. (Turnquist et al., 2007) demonstrated slowed tumor growth and increased numbers of DC and T cells clustered together as well as distributed through the tumor by intratumoral injection of SLC into tumor-burdened mice. Eo et al. (Eo et al., 2001) reported that SLC could promote T cell proliferation and increase the production of Th1-type cytokines (IFN-γ and IL-2). The anti-tumor effect of SLC has been demonstrated in many trials, but little has been done to investigate the feasibility of the combination of TAA and SLC for anti-tumor immunotherapy. In the present study, we modified human DCs with MUC1 or SLC by gene transduction of LVs for the first time to investigate their ability to stimulate anti-tumor CTLs in vitro. Four tumor cell lines including HCT-116, SGC-7901, MKN-28 and LOVO were analyzed as target cells for cytotoxic activity. We observed obvious CTL activity targeting HCT-116 cells, which is both MUC1-positive and HLA-A2-positive, induced by both the LV MUC1 DCs and LV SLC-MUC1 DCs. Meanwhile, no specific cytolytic activity was detected against MUC1-negative or HLA-A2-negative tumor cells in any of the groups. The cytolytic effect of CTLs induced by LV SLC-MUC1 DC was notably stronger than that of LV MUC1 DC. Furthermore, an enhanced capacity for T cell stimulation and an increased number of IFN-γ-secreting CTLs were induced by the SLC-MUC1 transduced DCs. These results suggest that the enhanced induction of anti-tumor CTLs by SLC might be due to its attraction of T cells to the site of DCs and thus promoted the interaction between DCs and T cells , consistent with the finding by Li et al. (Li et al., 2009). Besides, the increased IFN-γ secretion and proliferation of antigen-sensitized T cells activated by SLC also contributed to the enhancement of MUC1-specific antitumor immune responses. Effective exposure of naïve CD8+ T cells to DCs facilitated tumor antigen presentation and thus activation of naïve CD8+ T cells and priming of effective immune responses.

In summary, our study demonstrated that DCs could be effectively transduced with LVs without altering their maturation. MUC1 transduced DCs elicited effective anti-tumor responses in an antigen-specific and HLA-restricted manner, and the fusion of SLC to MUC1 could enhance these responses in vitro. Although additional studies are needed in vivo to verify these findings, our study suggests that the combination of MUC1 and SLC may be of potential value in DC-based anti-tumor immunotherapy.

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