REVIEW

# Genetic modification of dendritic cells and its application for cancer immunotherapy

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**Abstract: Dendritic cells (DCs) are the most potent antigen-presenting cells (APCs). DCs pulsed with peptides of tumor-associated antigens (TAA) and tumor lysate have been used in cancer immunotherapy. An early clinical study demonstrated the safety of the use of DCs, but the clinical response was not sufficient. The gene-modification of DCs with TAA and soluble factor genes such as cytokine and chemokine genes has been examined to enhance the antigen-presenting capacity of DCs. Viral vectors including retroviruses and adenoviruses have been reportred to be useful to obtain a sufficient transduction efficiency into DCs. TAA gene-transduced DCs could have several advantages compared with TAA peptide-pulsed DCs as follows : 1) The use of TAA gene-modified DCs are not restricted by MHC haplotypes. 2) The gene transduction with TAA genes is likely to present the unknown TAA peptides on DCs. 3) The gene-modified DCs show the prolonged presentation of TAA peptides. The transduction of DCs with cytokine genes including IL-12 and GM-CSF have also been reported to augument the antitumor effects of DCs. Although the results in the experimental systems were promising, the clinical application of gene-modified DCs includes several problems such as the standardization of methods of manipulation and gene-transduction of DCs. Approaches to solve them require further studies. J. Med. Invest. 49 : 7-17, 2002**

***Keywords*** **:** *dendritic cells (DCs), tumor-associated antigens (TAA), cytokine, chemokine, gene transduction, viral vector*

## INTRODUCTION

Dendritic cells (DCs) are the most potent antigen-presenting cells (APCs), which distribute in most tissues, capture antigens *in situ* and migrate to lymphoid organs to activate naive T cells (1, 2). In 1973, Steinman reported the novel cell type in murine spleen that shows the typical phenotype with long dendrites, and named them dendritic cells (3). Since the number of DCs, however, were very few at 1.0-1.6% in the spleen or less in other major organs including peripheral blood, it has been difficult to study the *in vitro* and *in vivo* functions of DCs. Since 1992, when the culture methods to generate DCs from monocytes and $CD34^+$ hematopoietic progenitor cells with cytokines *in vitro* were established (4-6), both basic and clinical research have rapidly progressed. Based on the analysis of DC functions, its clinical application for several diseases, especially for malignant diseases, has been performed using DCs pulsed with peptides and proteins of tumor-associated antigens (TAAs) or tumor lysate (7-10). Tumor cell-dendritic cell hybrids were also used for the treatment of renal cell carcinoma (11). These early clinical studies demonstrated the safety of DC-based immunotherapy, but the clinical responses were not so sufficient irrespective of some objective responses. To improve the antitumor effects in humans, the novel approaches or the combination

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with other modalities should be examined. One of the most potent approaches to enhance the APC function of DCs could be the genetic modification of DCs with antigen genes (12). In fact, vaccination with gene-modified DCs was more effective in suppressing tumor growth compared with vaccination with gene-modified tumor cells (13). The other genes including cytokines and chemokines have also been examined to augment immune responses against cancer. Here, we review the recent progresses in the study of gene-modified DCs.

## Efficient gene transfer into DCs

To modify DCs with foreign genes, the various methods of gene transfer have been examined (Table 1). The transduction markers such as LacZ and luciferase

Table 1. Transduction efficiency into DCs with various methods

| Method | Source of DC | Marker gene | Efficiency (%) | References |
|---|---|---|---|---|
| (1) Non-viral method | | | | |
| ①$CaPO_4$ | monocyte | luciferase | not detected | (17) |
| ②Liposomes | | | | |
| lipofectin | monocyte | LacZ | not described | (16) |
| lipofectAMINE, DOTAP | monocyte | luciferase | low | (17) |
| LipofectACE, lipofectin | | | | |
| LipofectAMINE | monocyte | GFP | 5% | (20) |
| ③Electroporation | monocyte | luciferase | low | (17) |
| | human CD34 | GFP | 12% | (19) |
| | monocyte | | ≦2% | |
| ④Gene Gunn | monocyte | luciferase | 5-10% | (66) |
| ⑤receptor mediated | | | | |
| transferring | mBM | LacZ, CAT | <5-10% | (21) |
| mannose | monocyte | GFP | 9-10% | (22) |
| (2) Viral method | | | | |
| ①Retrovirus | | | | |
| | monocyte | LacZ | 35-67% | (26) |
| | monocyte | LacZ | <30% | (30) |
| coculture with producer | human CD34 | mCD80 | 22-28% | (23) |
| coculture with producer | human CD34 | CD 2 | 11.5-21.2% | (27) |
| centrifugation | human CD34 | MUC-1 | <15% | (24) |
| DOTAP | human CD34 | GFP | <50% | (28) |
| coculture with producer | mBM | LacZ | 42-72% | (25) |
| centrifugation | mBM | GFP | 52-86% | (34) |
| centrifugation | mBM | EGFP, hCD80 | 22-75% | (32) |
| ②Adenovirus | | | | |
| | monocyte | LacZ | 95% (MOI 1000) | (17) |
| | monocyte | LacZ,GFP | >90% (MOI 100) | (42) |
| | DC line | LacZ | 33% (MOI 1000) | (36) |
| | mBM | LacZ | ≧80% (MOI 100) | (37) |
| | mBM | LacZ | 95% (MOI 100) | (38) |
| | mBM | EGFP | ≧90% (MOI 500) | (39) |
| LipofectAMINE | monocyte | GFP | ≧90% (MOI 50) | (20) |
| Fab-anti-CD40 | monocyte | GFP | 80% (MOI 100) | (41) |
| Centrifugation | monocyte | EGFP | 86% (MOI 50) | (40) |
| ③Lentivirus | | | | |
| | monocyte | EGFP | 70-90% | (44) |
| | human CD34 | EGFP | | (45) |
| ④Adeno-associated virus (AAV) | | | | |
| | monocyte | GFP | 2-55% | (46) |
| ⑤Influenza virus | | | | |
| | monocyte | GFP | ≧90% (MOI 1) | (47) |
| ⑥Avipox virus | | | | |
| | monocyte | CEA | 75% (MOI 30) | (77) |

mBM : mouse bone marrow, LacZ : β-galactosidase, GFP : green fluorescent protein, EGFP : enhanced GFP, MOI : multiplicity of infection

were used in early studies, whereas recent projects to examine the transduction of DCs employeed the green fluorescent protein (GFP) that spontaneously emitted green light without the substrate in living cells (14) (Figure 1). Since DCs are terminally differentiated and not dividing cells (15), it is difficult to transduce the foreign gene into DCs. First, Alijagic *et al.* reported the gene modification of human monocyte-derived DCs using liposome-mediated transduction of the tyrosinase gene (16). Although they found the proliferation of tyrosinase-specific T cells, the transduction efficiency determined by LacZ as a marker gene was too low to evaluate. Similarly, approaches using non-viral systems could not produce a high transduction efficiency, being about 10% at most (17-22). On the other hand, viral vectors, particularly retroviral and adenoviral vectors were used for the transduction of DCs in most of the studies reported recently. The retroviral transduction is limited to use with $CD34^+$ cell-derived DCs in humans and bone marrow-derived DCs in mice because of the requirement for cell proliferation. Furthermore, the additional techniques and repeated transduction were needed for the retroviral system to yield high efficiency since the conventional method of retroviral transduction could not produce high transduction efficiency. The co-culture of DCs with producer cells or the combination of centrifugation or liposome reported to be effective to enhance the transduction efficiency (23-35). In summary, the transduction efficiency by the retrovirus system varied was reported to be 11.5-86% (Table 1). On the other hand, the other viral vector that was commonly used for DC transduction was an adenoviral system, which was known to infect non-dividing cells with a high efficiency. To date, the adenovirus could be the most useful vector to transduce DCs with foreign genes since most findings using adenoviral transduction showed an efficiency greater than 80% (Table 1) (16, 19, 36-43). Furthermore, there appears to be several advantages in the adenoviral vectors : 1) the adenoviral vector is not integrated into host genomes when compared with a retroviral system. 2) most humans have an anti-adenoviral immunity, which might prevent the adverse effects caused by adenoviral vectors. However, even when used in an adenoviral system, DCs were relatively resistant to gene-modification compared with tumor cells. The combined use including liposomes and centrifugation was recommended to achieve a high transduction efficiency (20, 40). Recently, other viral systems such as Lentivirus (44, 45), adeno-associate virus (AAV) (46), influenza virus (47) and pox virus (48) were also reported to be useful for the gene-modification of DCs.

## Candidate genes for gene transduction of DCs

The candidate genes that have been tested for the gene-modification of DCs are described in Table 2. The effects of gene-tansduction of DCs with TAA genes were examined first. Recent studies reported findings with other genes including cytokines, chemokines and costimulatory molecules.

### *(1) Tumor-associated antigen (TAA) genes*

In 1991, Boon *et al.* first reported the successful cloning of the TAA gene which is specifically recognized by cytotoxic T lymphocytes (CTLs), and named it MAGE (49). Futhermore, they identified the antigenic peptides of MAGE-3, which were presented on MHC class I of APC and could induce antigen-specific $CD8^+$ CTLs (50, 51). Kawakami *et al.* also reported the melanocyte-specific antigens MART-1 and gp100 which were recognized by tumor-infiltrating lympho-

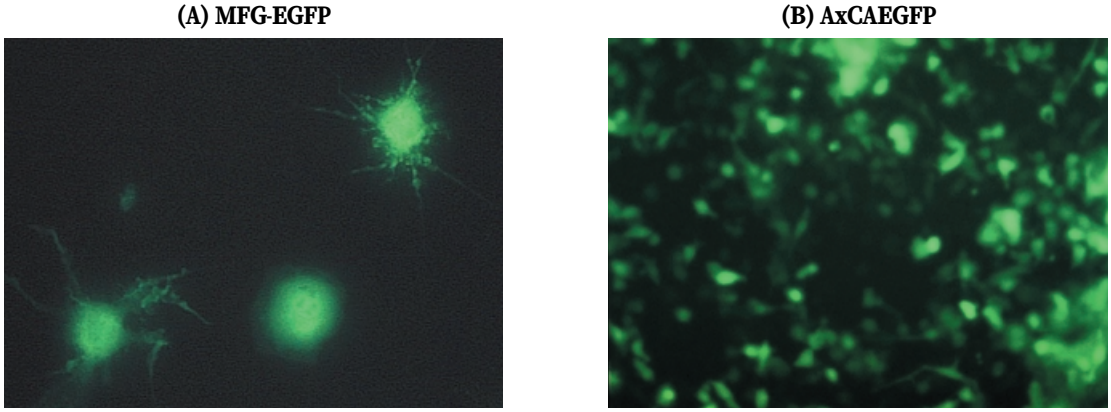


Figure. 1 EGFP (enhanced green fluorescent protein) gene-transduced mouse bone marrow- and human monocyte-derived dendritic cells. Mouse bone marrow-derived DCs were transduced with retrovirus MFG-EGFP (A). Human monocyte-derived DCs were transduced with adenovirus AxCAEGFP (B). These DCs were analyzed under a fluorescent microscope.

**Table 2. Effects of Gene-modified DCs**

| Genes | Source of DCs | Biological effects | References |
|---|---|---|---|
| (1) Antigen | | | |
| LacZ | mBM | CTLs (↑), Metastases (↓) | (61) (62) |
| OVA | mBM, mDC line | CTLs (↑), Tumor growth (↓) | (34) (36) |
| tyrosinase | monocyte | Growth of CTL line (↑) | (16) |
| MART-1, gp100 | human CD34 | CTLs (↑), | (23) |
| MART-1, gp100 tyrosinase, MAGE-1,3 | monocyte | CTLs (↑), | (67) |
| MART-1 | monocyte | CTLs (↑), | (63) (64) |
| MUC-1 | human CD34 | CTLs (↑), | (24) |
| MUC-1 | mBM | CTLs (↑), Tumor growth (↓) | (37) |
| p53 | mBM | CTLs (↑), Tumor growth (↓) | (18) (67) |
| AFP | monocyte | CTLs (↑), | (65) |
| AFP | mBM | CTLs (↑), Tumor growth (↓) | (66) |
| TRP-2 | mBM | CTLs (↑), Tumor growth (↓) | (39) |
| (2) Cytokines, chemokines | | | |
| IL-7 | monocyte | MLR (↑) | (30) |
| IL-12,IFN-α | monocyte | CTLs (↑), | (67) |
| IL-12 | mBM | CTLs (↑), Tumor growth (↓) | (32) |
| IL-12 | monocyte | MLR (↑) | (76) |
| GM-CSF | mBM | CTLs (↑), Tumor growth (↓) | (21) |
| lymphotactin | mBM | CTLs (↑), Tumor growth (↓) Metastases (↓) | (77) |
| (3) Cell surface molecules | | | |
| CD40 L | mBM | CTLs (↑), Tumor growth (↓) | (78) |
| CD80 | monocyte | CTLs (↑), IFN-γ (↑) | (79) |
| CD80, CD54, CD58 | monocyte | CTLs (↑), IFN-γ (↑) | (48) |

mBM : mouse bone marrow, CTLs : cytotoxic T lymphocytes, MLR : mixed leukocyte reaction, LacZ : β-galactosidase, OVA : ovalbumin, AFP : α-fetoprotein

cytes in melanoma (52, 53). These findings allowed us to start tumor vaccine therapy using TAA peptides. The early clinical studies for patients with metastatic melanoma using TAA peptides mixed with an adjuvant showed the induction of tumor specific immune responses and some objective responses (54, 55).

On the other hand, it was reported that the administration of DCs pulsed with TAA peptides was more effective in regressing established tumors than TAA peptides alone (56, 57). Many investigators have now focused on the use of DCs for cancer immunotherapy to obtain better clinical effects. In addition to the use of TAA peptides or tumor lysate, fusion of DCs with tumor cells (58), pulsing with tumor RNA (59), exosomes (60) and the gene-modification of DCs (12) have been reported to be hopeful strategies. Among them, one of the best use of DCs could be the gene-transduced DCs with the TAA gene due to the following possibilities : 1) The use of TAA gene-modified DCs is not restricted by MHC haplotypes. 2) The gene transduction with TAA genes is likely to present unknown TAA peptides on DCs. 3) The gene-modification prolongs the presentation of TAA peptides on DCs. In the early experiments, the tumor cells modified to express foreign antigens such as β-galactosidase (β-Gal) and ovalbumin (OVA) have been used (34, 36, 61, 62). However, since these artificial antigens have shown a strong immunogenicity that is different from that of endogenous TAA, experiments with endogenous TAA are necessary before initiating clinical trials of immunotharapy against human cancers. To answer this, Kaplan *et al*. demonstrated that therapy with DCs transduced with endogenous TAA antigen TRP (tyrosinase-related protein)-2 effectively induced the tumor-specific immunity and regressed B16 tumors (39). This observation could be important since they first demonstrated the possibility that immunization with endogenous TAA, in which immunogenicity was presumably low, was also effective for inducing tumor-specific immunity and inhibiting tumor growth. In humans, Reeves *et al*. reported MART-1 gene transduction of human $CD34^+$ cell-derived DCs with retrovirus system and the induction of CTLs specific for MART-1 *in vitro* (23). Butterfield *et al*. also demonstrated the MART-1 gene-modification of human monocyte-derived DCs with adenoviral vector and the effective CTL induc-

tion using gene-modified DCs (63, 64). They next reported the efficient induction of CTLs specific for α-fetoprotein (AFP) by AFP-transduced DCs as an immunotherapy for patients with hepatocellular carcinoma (65, 66). The DCs modified to express other TAA genes including p53, MAGE-1, 3 and MUC-1 have been tested for their ability to induce the antigen-specific CTLs *in vitro* (18, 24, 37, 48, 67, 68). Although the gene-modified DCs with these TAA genes have been effective in generating CTLs *in vitro*, it is still unclear what type of TAA genes are most effective for what types of cancer.

*(2) The cytokine and chemokine genes*

Various cytokines and chemokines were involved in the process of antigen presentation and CTL induction by DCs (1). Interleukin (IL)-12 enhances NK cell and CTL activities, plays a key role in the induction of Th1 immune responses including IFN-γ production (69), and promotes the growth of T and NK cells (70, 71). The administration of IL-12 protein and IL-12 gene-transduction into tumor cells has shown profound antitumor effects (72, 73). Granulocyte-macrophage colony stimulating factor (GM-CSF) is known to be an essential cytokine to generate DCs from both bone marrow cells and monocytes and stimulate the survival of DCs (4-6). For these reasons, studies regarding the transduction of DCs with cytokine genes were initially examined using IL-12 and GM-CSF genes. Melero *et al*. and we demonstrated that the intratumoral injection of IL-12 gene-transduced DCs induced the tumor specific immune responses and regressed the established tumors in mice (32, 74). The antitumor effects of intratumoral injection of IL-12 gene-modified DCs were found to be better than that of IL-12 gene-modified fibroblasts that have been used in clinical trial as a phase I and II study (32). GM-CSF gene-modified DCs pulsed with TAA peptides were also demonstrated to be more effective in inducing antitumor immunity than nontransduced DCs (21). It was suggested that these effects were mediated by the increased survival and migration to draining lymph nodes (21). The approaches of intratumoral injection were applied for IL-7 gene-modified DCs (75). They compared IL-7 gene-modified DCs with TAA-loading DCs, and found DC-IL-7 to be as effective as TAA-loaded DCs and superior to tumor lysate-pulsed DCs (75). Even when human DCs were transduced with IL-12 and IL-7 genes, these cytokine gene-transduced DCs showed the enhancement of the allogeneic MLR, indicating that these approaches could be applicable for humans (30, 76)

Chemokines would also be better candidates to enhance immune responses *in vivo*. The transduction of the lymphotactin gene was examined using combinations with TAA peptides (77). The vaccination of DC-lymphotactin pulsed with TAA peptide was more effective in inducing specific antitumor immunity and reducing lung metastases of 3LL tumors when compared with nontransduced DCs (77).

*(3) The cell surface molecules*

CD40L is a costimulatory molecule that is expressed on activated $CD4^+$ T cells and stimulates APCs through the CD40-CD40L interaction (1). To activate DCs directly, Kikuchi *et al*. transduced the CD40L gene to murine DCs and evaluated the antitumor effects of CD40L gene-modified DCs (78). Infection of DCs by AdCD40L induced IL-12 and MIP-1α productions, and the intratumoral administration of CD40L-transduced DCs induced the regression of pre-existing tumors (78).

DCs express CD80 molecules, but the level of CD80 expression is not high. Tsang *et al*. examined CD80 gene transduction into human DCs and found that the CD80 gene-modification of DCs enhanced IFN-γ production and cytotoxicity of antigen-specific CTLs using CEA-specific T cells (79). They extended this study and reported the imunostimulatory effect of transduction of three costimulatory molecules (CD80, CD54 and CD58) using avipox virus (48). The human DCs transduced with a triad of costimulatory molecules significantly generated peptide-specific CTLs *in vitro* (48). It might be a great advantage that the avipox viral vector could express three transgenes on human DCs at the same time.

## Future perspectives and problems with the application of gene-modified DCs

The schema of DC-based cancer immunotherapy in humans is shown in figure 2. There have been three types of human DCs used in the clinical trials such as monocyte-derived, $CD34^+$ cell-derived and blood DCs. Among them, the monocyte-derived DCs are convenient to use in clinics due to their ease in preparation. However, the standard method of DC-based immunotherapy has not yet been established. There are some problems that needed to be clarified to optimize DC therapy as follows : 1) the kind of DCs best for tumor immunotherapy 2) the optimal protocol of DC administration 3) the most effective TAA. To date, some studies have answered these questions. For ex-

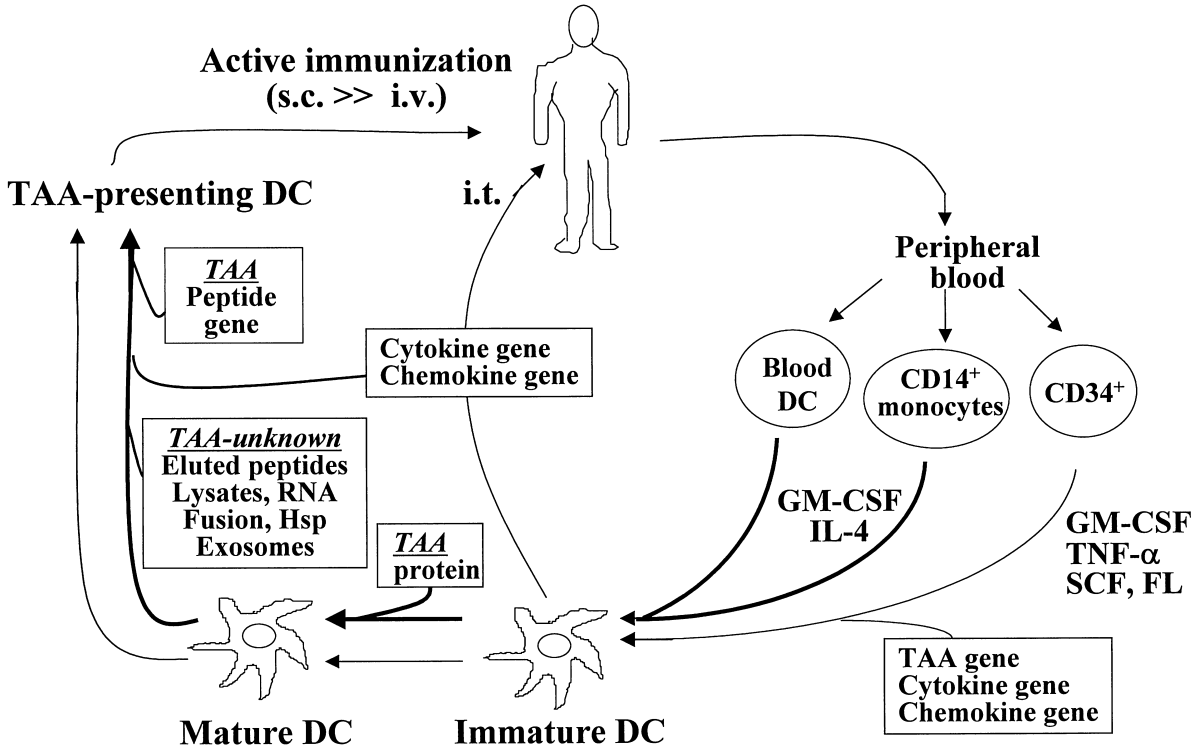


**Figure. 2** Cancer immunotherapy using dendritic cells
(SCF : stem cell factor, FL : flt3 ligand, s.c. : subcutaneous, i.v. : intravenous, i.t. : intratumoral)

ample, the findings reported by Eggert *et al.* showed that the s.c. injection of DCs was better than the i.v. injection for inducing the antitumor immunity in mice (80). Mores *et al* . demonstrated that the migration of DCs into lymph nodes was much better after s.c. injection when compared with i.v. route in humans (81). Based on these observations, the s.c. injection is suggested to be the most useful route for DC administration. Furthermore, the comparative studies on the function between monocyte-derived and $CD34^+$ cell-derived DCs have been reported. Mortarini *et al.* and Felazzo *et al.* showed that $CD34^+$ cell-derived DCs were more potent to induce CTLs than monocyte-derived DCs (82, 83). However, further studies are required to clarify whether $CD34^+$ cell-derived DCs are better than monocyte-derived DCs for cancer immunotherapy. The subset of DCs (84) and the novel findings of DC function such as the interaction with innate immunity (85, 86) and the trafficking capacity (87) should be also considered to establish the standard protocol of DC-based immunotherapy. Since the gene-modified DCs have shown strong antitumor effects against various types of tumors in animal models, future studies would be expected to lead to clinical trials.

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