*Original Article*

# HER2-Specific T Lymphocytes Kill both Trastuzumab-Resistant and Trastuzumab-Sensitive Breast Cell Lines *In Vitro*

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## ABSTRACT

**Objective:** Although the development of trastuzumab has improved the outlook for women with human epidermal growth factor receptor 2 (HER2)-positive breast cancer, the resistance to anti-HER2 therapy is a growing clinical dilemma. We aim to determine whether HER2-specific T cells generated from dendritic cells (DCs) modified with *HER2* gene could effectively kill the HER2-positive breast cancer cells, especially the trastuzumab-resistant cells.

**Methods:** The peripheral blood mononuclear cells (PBMCs) from healthy donors, whose HLA haplotypes were compatible with the tumor cell lines, were transfected with reconstructive human adeno-association virus (rhAAV/*HER2*) to obtain the specific killing activities of T cells, and were evaluated by lactate dehydrogenase (LDH) releasing assay.

**Results:** Trastuzumab produced a significant inhibiting effect on SK-BR-3, the IC50 was 100ng /ml. MDA-MB-453 was resistant to trastuzumab even at a concentration of 10,000 ng/ml *in vitro*. HER2-specific T lymphocytes killed effectively SK-BR-3 [(69.86±13.41)%] and MDA-MB-453 [(78.36±10.68)%] at 40:1 (effector:target ratio, E:T), but had no significant cytotoxicity against HER2-negative breast cancer cell lines MDA-MB-231 or MCF-7 (less than 10%).

**Conclusion:** The study showed that HER2-specific T lymphocytes generated from DCs modified by rhAAV/HER2 could kill HER2-positive breast cancer cell lines in a HER2-dependent manner, and result in significantly high inhibition rates on the intrinsic trastuzumab-resistant cell line MDA-MB-453 and the tastuzumab-sensitive cell line SK-BR-3. These results imply that this immunotherapy might be a potential treatment to HER2-positive breast cancer.

**Key words:** HER2-positive breast cancer; Trastuzumab-resistant; Dendritic cells; Immunotherapy; Reconstructive human adeno-association virus

## INTRODUCTION

Breast cancer is the most frequently diagnosed cancer and the leading cause of cancer death among females[1]. Human epidermal growth factor receptor 2 (HER2) over-expression or *HER2* gene amplification exists in about 25% to 30% of breast cancer, which is called HER2-positive breast cancer representing a distinct disease entity confirmed by gene expression profiling studies[2-4]. *HER2* gene amplification is also an independent poor prognostic factor, which is associated with more aggressive, high risk of recurrence and poor prognosis characterized by resistance to traditional systemic therapy[5-8]. Although the development of trastuzumab and lapatinib has improved the outlook for women with HER2-positive disease, resistance to anti-HER2 therapy is a growing clinical dilemma[9,10].

With the progresses in immunology, immunotherapy has become an important means of cancer treatment. Since HER2 is over-expressed at the cell surface of tumor cells, it represents a good target for anti-cancer immunotherapy[11]. Currently, anti-HER2 immunotherapy researches focus on anti-HER family antibodies, anti-HER2 vaccines and adoptive immunotherapy of cancer using HER2 antigen-specific T lymphocytes [cytotoxic T lymphocytes (CTL)

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mainly][10,12]. Dendritic cells (DCs) are the most powerful antigen presenting cells (APCs). They also play an important role in tumor immune escape and tolerance mechanism, including the existence of distinct DC subsets that respond differentially to distinct activation signals and inability of the antigen-presenting function. To overcome this problem, genetic modification of DCs *in vitro* can be aimed at expressing tumor antigens or changing their biology to increase their ability to present antigens, taking advantage of the high efficiency of antigen presentation and T cell activation by DCs[13,14]. Reconstructive human adeno-association virus (rhAAV) has the ability to infect both non-dividing and dividing cells with persistent expression which made it an attractive gene transfer vector. AAV vectors are one of the safest for use in gene therapy and high effective for the delivery of antigens and cytokine genes into human DCs. They do not show directly toxic to DCs or induce a cytopathic effect in DCs, and they may help the process of DC's maturation[15]. The genetic manipulation of DCs by rhAAV vector-deliveries of self antigen may circumvent and surmount innate immune tolerance. Using rhAAV as vectors, autologous DCs could be loaded by *HER2*, prostate-specific antigen *(PSA)* and other tumor antigen genes. These modified DCs can induce specific anti-tumor immunity against tumor cells carrying the target antigen[16,17].

It is still unknown that whether tumor antigen-specific T cells generated by DCs pulsed with *HER2* cDNA could attack trastuzumab-resistant HER2-positive breast cancer cell lines. We aim to determine whether HER2-specific T cells generated from human peripheral blood DCs transfected with rhAAV/*HER2* could effectively kill the both HER2-positive breast cancer cell lines *in vitro*.

## MATERIALS AND METHODS

### Generation of rhAAV/*HER2* Viruses

Three rhAAV/*HER2* plasmids were kindly provided by Dr. Linda Santschi (Coastal Marine Biolab, USA). The partial *HER2* cDNA sequences, ligated into the pAAV-MCS, encoded amino acids 153–653 (left, HER2-L), 403–906 (middle, HER2-M), and 796–1,255 (right, HER2-R), respectively. The three rhAAV/*HER2* vectors were generated by using the AAV Helper-Free System (Stratagene), according to manufacturer's instructions. Virus stock was titered to determine vector genomes per ml (v.g./ml) by using the QuickTiter™ AAV Quantitation Kit's (Cell biolabs), yielding titers of approximately $1\times10^{10}$ v.g./ml.

### Cell Lines and HER2 Expression Analysis Detected by Immunocytochemistry (IHC)

The human breast cancer cell lines MDA-MB-453 and SK-BR-3 were kindly given by Professor Liu Zhihua (Cancer Institute and Hospital, Chinese Academy of Medical Sciences), the cell lines MDA-MB-231 and MCF-7 were obtained from the cell culture center of Institute of Basic Medical Science, Chinese Academy of Medical Sciences (Beijing, China). All the cell lines were maintained in RPMI 1640 with 4% fetal bovine serum (FBS, GIBCO, USA), 100 U-100 μg/ml penicillin-streptomycin (GIBCO), in 75 $cm^2$ plastic flasks (Coring, USA) at 37°C in a humidified atmosphere with 5% $CO_2$. Cells were harvested by 0.25% trypsin-ethylene diamine tetraacetic acid (EDTA, GIBCO). All experiments were done with subconfluent cells in the exponential phase of growth.

The breast cancer cells were then stained with anti-HER2/neu (4B5) monoclonal antibodies (Ventana) as previously described[18]. IHC scoring system according to the guidelines given by American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP)[18].

### Generation of DCs Infected by rhAAV/*HER2* and Activated T Lymphocytes

The peripheral blood mononuclear cells (PBMCs) from healthy donors were separated by routine ficoll gradient method. All blood donors were given informed consent in writing. The human leukocyte antigen (HLA)-A or HLA-B haplotype, (HLA-A*/B* SSP Kits, Protrans, Germany) of donors was respectively compatible with one of the HER2-positive cell lines (MDA-MB-453, SK-BR-3) and one of the HER2-negative cell lines (MDA-MB-231, MCF-7). After cultured for 4 h in AIM-V medium, the non-adherent cells were transferred to 6-well culture plates in AIM-V containing interleukin-2 (IL-2, Tebao, Xiamen, China, 20 IU/ml). The adherent monocytes were infected immediately with $10^5$ v.g./cell (multiplicity of infection, MOI) rhAAV/*HER2* (rhAAV/*HER2*-L, rhAAV/*HER2*-M, rhAAV/*HER2*-R, together) and cells without transfection were set as control. After 4 h, the medium/virus solution was removed and the cells were finally fed with AIM-V medium containing recombinant human granulocyte-macrophage colony stimulating factor (GM-CSF) (200 ng/ml) and recombinant human IL-4 (R & D Systems, USA, 10 ng/ml). On day 6, tumor necrosis factor-α (TNF-α, R & D Systems, USA, 100 IU/ml) was added to the medium. The medium and cytokines were replaced every two days. On day 3, day 5 and day 7, the culture supernatants were collected, IL-10 and IL-12 secretion was measured in duplicate by enzyme-linked immune sorbent assay (ELISA) using commercially available kits (BOSTER, China), according to the manufacturer's instructions.

On day 7, the mature DCs were harvested and mixed with peripheral blood lymphocytes (PBLs) (PBL:DC ratio, 20:1), keeping the two groups: the rhAAV/*HER2* transfected group, and the non-rhAAV/