Vaccine 36 (2018) 1414–1422

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Heterologous human/rat HER2-specific exosome-targeted T cell vaccine stimulates potent humoral and CTL responses leading to enhanced circumvention of HER2 tolerance in double transgenic HLA-A2/HER2 mice

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ARTICLE INFO

Article history:
Received 10 August 2017
Received in revised form 5 January 2018
Accepted 29 January 2018
Available online 5 February 2018

Keywords:
Heterozygous HER2
T cell vaccine
Antibody
CTL
Tolerance
Transgenic HER2 mice

ABSTRACT

DNA vaccines composed of heterologous human HER2 and rat neu sequences induce stronger antibody response and protective antitumor immunity than either HER2 or neu DNA vaccines in transgenic mice. We previously developed HER2-specific exosome-targeted T-cell vaccine HER2-T_{EXO} capable of stimulating HER2-specific $CD8^+$ T-cell responses, but only leading to partial protective immunity in double-transgenic HLA-A2/HER2 mice with self-immune tolerance to HER2. Here, we constructed an adenoviral vector AdV_{HuRt} expressing HuRt fusion protein composed of NH_2-$HER2_{1-407}$ (Hu) and COOH-$neu_{408-690}$ (Rt) fragments, and developed a heterologous human/rat HER2-specific exosome-targeted T-cell vaccine HuRt-T_{EXO} using polyclonal $CD4^+$ T-cells uptaking exosomes released by AdV_{HuRt}-transfected dendritic cells. We found that the HuRt-T_{EXO} vaccine stimulates enhanced $CD4^+$ T-cell responses leading to increased induction of HER2-specific antibody (~70 μg/ml) compared to that (~40 μg/ml) triggered by the homologous HER2-T_{EXO} vaccine. By using PE-H-$2K^d$/$HER2_{23-71}$ tetramer, we determined that HuRt-T_{EXO} stimulates stronger HER2-specific $CD8^+$ T-cell responses eradicating 90% of HER2-specific target cells, while HER2-T_{EXO}-induced $CD8^+$ T-cell responses only eliminating 53% targets. Furthermore, HuRt-T_{EXO}, but not HER2-T_{EXO} vaccination, is capable of suppressing early stage-established HER2-expressing $4T1_{HER2}$ breast cancer in its lung metastasis or subcutaneous form in BALB/c mice, and of completely protecting transgenic HLA-A2/HER2 mice from growth of HLA-A2/HER2-expressing $BL6\text{-}10_{A2/HER2}$ melanoma. HuRt-T_{EXO}-stimulated HER2-specific $CD8^+$ T-cells not only are cytolytic to trastuzumab-resistant HLA-A2/HER2-expressing BT474/A2 breast tumor cells *in vitro* but also eradicates pre-established BT474/A2 tumors in athymic nude mice. Therefore, our novel heterologous human/rat HER2-specific T-cell vaccine HuRt-T_{EXO}, circumventing HER2 tolerance, may provide a new therapeutic alternative for patients with trastuzumab-resistant $HER2^+$ breast tumor.

1. Introduction

The human epidermal growth factor receptor (EGFR) 2 (HER2) is a receptor tyrosine kinase, belonging to the EGFR group [1]. It is over-expressed in breast cancer especially the ductal carcinoma with poor prognosis [1]. The human HER2 molecule is of 84% amino acid sequence homology to the rat neu protein of rat neuroblastoma [2,3]. The humanized anti-HER2 antibody trastuzumab (Herceptin) is effective in treating HER2-positive breast cancer showing an objective response rate of 12–34% [4]. However, patients often develop resistance against trastuzumab [5,6], possibly due to the compensatory activities *via* alternative signaling pathways [7,8]. It has also been reported that a risk of cardiac

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https://doi.org/10.1016/j.vaccine.2018.01.078
0264-410X/

toxicity [9,10] limits the clinical use of trastuzumab [5,6], warranting the search for other active therapies [11].

Tumor-specific $CD8^+$ cytolytic T lymphocytes (CTLs) recognizing tumor antigenic peptide/major histocompatibility complexes-I (pMHC-I) presented on tumor cells play an important role in antitumor immunity [12]. However, immune tolerance including central (natural) or peripheral (acquired) tolerance becomes a major obstacle in antitumor immunity [13]. Central tolerance refers to the tolerance established in the thymus by deleting high affinity autoreactive $CD8^+$ T cells and differentiating some autoreactive T lymphocytes into natural polyclonal $CD4^+CD25^+Foxp3^+$ or $CD8^+$-$CD25^+Foxp3^+$ regulatory T (nTreg) cells, which act as sentinels to suppress potential T cell autoreactivity [14]. Peripheral tolerance is driven by induced Treg (iTreg) cells such as $CD4^+Fop3^+$ Treg and $CD4^+Foxp3^-$ type-1 Treg (Tr1) or type-3 helper T (Th3) cells developed in peripheral lymphoid tissues or organs. These cells have critical role in mucosal immune tolerance and controlling allergic inflammation, and become major obstacles in antitumor immunity [14].

Heterologous antigens that share a significant sequence homology with self-antigens have been shown to be effective in overcoming peripheral tolerance [15], triggering autoimmune diseases, such as mouse experimental autoimmune encephalomyelitis and arthritis, respectively, by using bovine myelin basic protein [16] and bovine, porcine or human collagen [17]. Tumor antigens detected in sera of cancer patients are often self-tolerated proteins inducing central and peripheral immune tolerance [18,19]. To break the tolerance, heterologous DNA vaccines composed of both human HER2 and rat neu DNA sequences have been shown to stimulate stronger antibody responses and more efficient antitumor immunity than either HER2 or neu DNA vaccine in transgenic (Tg) mice with HER2-specific self-immune tolerance [3,20]. However, the HER2-specific therapeutic antitumor immunity derived from immune cell-based vaccines engineered to express heterologous human HER2 and rat neu has yet been investigated.

We previously demonstrated that a recombinant adenoviral vector-mediated neu gene-engineered dendritic cell (DC) vaccine is superior to neu DNA vaccination by stimulating stronger neu-specific humoral and cellular immune responses in wild-type mice [21]. We also developed a novel ovalbumin (OVA)-specific T cell-based vaccine OVA-T_{EXO}, using ConA-stimulated polyclonal $CD4^+$ T cells with the uptake of OVA-specific dendritic cell (DC_{OVA})-released exosomes (EXO) *via* DC's CD54 and T cell's LFA-1 interaction [22,23]. Since the OVA-T_{EXO} cells expressed exosomal pMHC-I and CD80 and T cell's CD40L and IL-2, they could thus directly stimulated OVA-specific $CD8^+$ T cell responses [22,23]. We showed that the OVA-T_{EXO} vaccine induced more efficient OVA-specific CTL responses and antitumor immunity than the DC_{OVA} vaccine because it counteracts $CD4^+25^+Foxp3^+$ Treg suppression [22,23]. Recently, we developed a HER2-specific DC (DC_{HER2})-released EXO-targeted T cell-based vaccine, HER2-T_{EXO}, and demonstrated that the HER2-T_{EXO} vaccine was capable of stimulating HER2-specific CTL responses. However, it induced only partially protective immunity in double-Tg HLA-A2/HER2 mice with self-immune tolerance to HER2 [24]. Therefore, it would be interesting to determine, whether heterologous human/rat HER2-specific T cell vaccine enhances the circumvention of the HER2 tolerance in our double Tg HLA-A2/HER2 mice.

In this study, we constructed by recombinant DNA technology a recombinant adenoviral vector AdV_{HuRt} carrying a fused HuRt cDNA encoding human NH_2-$HER2_{1-407}$ (Hu) and rat COOH-$neu_{408-690}$ (Rt) fusion protein. With the help of this construct, we developed a heterologous human/rat HER2-specific EXO-targeted T cell-based vaccine HuRt-Texo. This was achieved by using polyclonal $CD4^+$ T cells with the uptake of human HER2/rat neu-specific EXO released by AdV_{HuRt}-transfected DCs. We then assessed enhancement of HER2-specific $CD8^+$ CTL responses and antitumor immunity in wild-type BALB/c and double Tg HLA-A2/HER2 mice immunized with HuRt-T_{EXO}. In addition, we also examined the cytolytic effect of HuRt-T_{EXO}-stimulated $CD8^+$ T cells against trastuzumab-resistant HER2-positive breast cancer cells, BT474, *in vitro*, and in BT474 breast cancer-bearing athymic nude mice *in vivo*.

2. Materials and methods

2.1. Antibodies, cell lines and animals

Phycoerythrin (PE)- or fluorescein isothiocyanate (FITC)-labeled anti-CD4, CD8 and CD44 antibodies (Abs) were obtained from Biolegend (San Diego, CA). PE-labeled H-$2K^d$/$HER2_{63-71}$ (TYLPTNASL) tetramer was obtained from Cedarlane (Burlington, Ontario, Canada). The humanized HER2-specific pertuzumab and trastuzumab (Herceptin) Abs were obtained from Genentech (San Francisco, CA). The mouse anti-HER2 TA-1 Ab was obtained from EMD Millipore (Darmstadt, Germany). The mouse neu-specific 7.16.4 Ab was obtained from Dr. Mark Greene, University of Pennsylvania, Philadelphia, PA) [25]. Tg1-1 is a neu-expressing mouse breast cancer cell line obtained from Dr. T Kipps, University of California, San Diego, CA [26]. HLA-A2/HER2-expressing $BL6\text{-}10_{A2/HER2}$ tumor cells were generated by transfection of mouse B16 melanoma BL6-10 cells with pcDNA-HLA-A2/pcDNA-HER2 in our lab [27]. Mouse breast cancer cell line 4T1 (H-$2K^d$) was obtained from Dr. J Hu, University of Toronto, Toronto, Ontario, Canada. HER2-positive human breast cancer MCF-7 and HER2-positive human trastuzumab-resistant BT474 breast cancer cell lines were obtained from American Type Tissue Collection (Rockville, MD) and Dr. S Kane (Beckman Research Institute of the City of Hope, Duarte, CA) [28], respectively. Female wild-type (WT) BALB/c (H-$2K^d$), transgenic (Tg) HLA-A2 and athymic nude mice were obtained from the Jackson Laboratory (Bar Harbor, MA). The Tg HER2 (H-$2K^b$) mice [29] with HER2-specific self-immune tolerance were obtained from Dr. W. Z. Wei (Wayne State University, Detroit, MI). The double Tg HLA-A2/HER2 mice were generated by backcrossing male HER2 mice with female HLA-A2 mice in our laboratory All mice were treated according to animal care committee guidelines of the University of Saskatchewan.

2.2. Recombinant adenoviruses

Recombinant $AdV_{HLA\text{-}A2}$ expressing HLA-A2 [24] as well as recombinant adenoviruses AdVneu [26] and AdV_{HER2} [27] expressing rat neu_{1-690} (XbaI/HindIII) and human $HER2_{1-690}$ (XbaI/HindIII) fragment, respectively, were previously constructed in our lab (Fig. 1A). Each neu_{1-690} or $HER2_{1-690}$ fragment includes a signal peptide (amino acids 1-21), an extracellular (amino acids 22-652), a trans-membrane (Tm, amino acids 653-675) and a partial intracellular (amino acids 676-690) domain (Fig. 1B). A pair of primers: XbaI, 5′-gctct agatg agcac catgg agctg-3′ (forward) and HindIII, 5′-gtaag ctttg atctc ttcca gagtc-3′ (reverse) and another pair of primers: HindIII, 5′-caaag cttac ctgta catct cagca-3′ (forward) and HindIII, 5′-agaag ctttc agcag cctac gcatc-3′ (reverse) were used to clone Hu gene fragment (XbaI/HindIII) and Rt gene fragment (HindIII/HindIII), respectively, by PCR. To construct heterologously fused gene HuRt, we cloned both human HER2 (encoding HER2 amino acids 1-407) ($HER2_{1-407}$ or short termed Hu) gene fragment with XbaI/HindIII ends and rat neu (encoding neu amino acids 408-690) ($Neu_{408-690}$ or short termed Rt) gene fragment with HindIII/