Novel EXO-T vaccine using polyclonal CD4+ T cells armed with HER2-specific exosomes for HER2-positive breast cancer

Abstract: Breast cancer is the leading cause of death in women globally. The human epidermal growth factor receptor 2 (HER2)-positive breast cancer is often associated with poor prognosis and high mortality. Even though anti-HER2 monoclonal antibodies have improved the clinical outcome, resistance to the antibody therapy becomes a major obstacle in the treatment of HER2-positive breast cancer patients. Alternative approaches are therefore needed. HER2-specific vaccines have been developed to trigger patient’s immune system against HER2-positive breast cancer. This article describes the development of novel HER2-specific exosome (EXO)-T vaccine using polyclonal CD4+ T cells armed with HER2-specific dendritic cell-released EXO and demonstrates its therapeutic effect against HER2-positive tumor in double-transgenic HER2/HLA-A2 mice with HER2-specific self-immune tolerance. Therefore, our novel HER2-specific EXO-T vaccines are likely to assist in the treatment of HER2-positive breast cancer patients.

Keywords: EXO-T vaccine, polyclonal CD4+, T cell, HER2, exosome, breast cancer

Introduction

Breast cancer is the most commonly diagnosed cancer and the second most common cause of cancer death in women in the USA.1–3 The human epidermal growth factor receptor 2 (HER2) oncogene encodes for a 185 kD transmembrane glycoprotein receptor with intracellular tyrosine kinase activity.4 It belongs to the human EGFR family including HER1, HER2, HER3, and HER4 that control breast cancer cell proliferation, migration, and invasion.5 Amplification of HER2 is observed in approximately 20% of human breast cancers.6–8 HER2-positive breast cancer is associated with increased rates of metastasis, reduced time to relapse, poorer prognosis, and higher mortality.6,9 Development of HER2-targeted immunotherapeutics such as HER2-specific monoclonal antibodies trastuzumab and lapatinib has greatly improved therapeutic outcome.10 Trastuzumab is remarkably effective both as monotherapy and in combination with cytotoxic chemotherapy in patients with HER2-positive metastatic breast cancer. However, most patients sooner or later develop resistance to trastuzumab during trastuzumab treatment,11,12 warranting the development of other effective HER2-targeted therapies.

Three signals in CD8+ T cell response

CD8+ cytotoxic T lymphocytes (CTLs) are responsible for adaptive immune responses against tumor. After antigen presentation to naive CD8+ T cells by antigen-presenting cells, such as dendritic cells (DCs), CD8+ T cells start to proliferate and become cytotoxic effectors capable of inducing cancer cell death via secreting cytokines (tumor necrosis factor-α and interferon-γ [IFN-γ]) and cytolytic granzyme-B.13 There are three conventional signals
participating in induction of CD8⁺ CTL immunity. The first signal is derived from the antigen peptide-presenting major histocompatibility complexes (pMHC-I) on DCs, which recognize the antigen-specific T-cell receptors (TCRs) on CD8⁺ T cells (Figure 1A). The second costimulatory signal is the interaction of DC’s costimulatory CD80 molecules with CD28 ligands on CD8⁺ T cells (Figure 1A). The third signal represents the innate inflammatory cytokines such as IL-12 and IFN-α stimulating CD8⁺ T cells (Figure 1A). The first two signals are responsible for naïve CD8⁺ T-cell proliferation, while IL-12 and IFN-α are in charge of the development of CTL effector functions. Apart from those signals, IL-15 secreted by DCs induces T-cell memory formation.

**Exosome-targeted polyclonal CD4⁺ T cell vaccine**

Some HER2-positive breast cancer patients have been found to develop spontaneous anti-HER2-specific immunity with both antibody and CD8⁺ T-cell responses, indicating that HER2 is an immunogenic target for the development
of anti-HER2 vaccines to stimulate patient’s own immune system against breast cancer. HER2-specific vaccines using HER2-specific peptides, proteins, DNA, or DCs have been developed, but mostly showing relatively limited antitumor effects.16

Exosomes (EXOs) are small vesicles of 50–100 nm in diameter secreted by budding from the cellular membrane.17 DC-released EXOs are enriched in immunological molecules important for DC’s stimulatory machinery.17 Similar to the previous adoptive engineered CD8+ T-cell therapy using active polyclonal CD8+ T cells engineered to express tumor-specific TCR,18 we developed novel CD4+ T-cell-based (EXO-T) vaccines using active polyclonal CD4+ T cells armed with tumor-specific DC-released EXOs.19–25 In the former one, polyclonal CD8+ T cells are genetically engineered to express tumor-specific TCRs containing signaling domain of CD3 zeta-chain or to express chimeric antigen receptor containing single-chain Fv fused to signaling domain of T-cell costimulatory molecules such as 41BB leading to the currently well-known chimeric antigen receptor-T therapy.26 In the latter one, EXO-T vaccines prepared by simply incubation of ConA-stimulated polyclonal CD4+ T cells with antigen-specific DC-released EXOs. The polyclonal CD4+ T cells took up antigen-specific DC-released EXOs via interaction of exosomal CD54 with T cell lymphocyte function-associated antigen 1, leading to the expression of exosomal surface molecules (pMHC-I and CD80) on CD4+ T cells via vesicle internalization/recycling and direct membrane fusion.19 As a result, the polyclonal CD4+ T cells phenotypically armed with antigen-specific exosomal pMHC-I complexes and exosomal CD80 molecule became antigen-specific EXO-T vaccines.19–25

Compared with vaccination of DCs presenting the three conventional signals, EXO-T vaccines stimulate CD8+ T-cell responses via three distinct signals namely 1) acquired exosomal pMHC-I, 2) acquired exosomal CD80 and CD4+ T cell CD40L, and 3) CD4+ T cell IL-2 (Figure 1B). EXO-T vaccines have been found to stimulate potent CD4+ T-cell-independent CTL responses19–25 and to promote CTL memory via CD4+ T cell CD40L signaling.20

CD8+ CTL exhaustion with overexpression of inhibitory molecules such as PD-1, Tim-3, and LAG-3 and with functional deficiency in the production of effector cytokine IFN-γ and effector cytolytic granzyme-B is a state of dysfunction that commonly occurs during cancer and infection diseases, which leads to failure in reducing viral or tumor load.27 We demonstrated that EXO-T vaccine was able to convert CTL exhaustion in chronic infection via CD4+ T cell CD40L signaling-induced activation of mTORC1 pathway, leading to CTL proliferation, IFN-γ production, and rescuing CTL cytotoxic effect.21 Because tumor-specific effector CTLs that undergo tumor tolerogenic microenvironment also become terminally differentiated into exhausted CTLs without any antitumor properties,28 our novel EXO-T vaccine may thus be able to exert its conversional effect on exhausted CTLs within tumors (Figure 1C).

**HER2-Texto vaccine**

We have recently developed Neu-specific (the rat’s form of human HER2) or HER2-specific EXO-T vaccines (Neu-Texto and HER2-Texto) using active polyclonal CD4+ T cells with uptake of Neu- or HER2-specific DC-released EXOs.22 We demonstrated that Neu-specific EXO-T vaccine stimulated Neu-specific CTL responses against Neu-expressing breast cancer Tg1-1 in transgenic FVBnueN mice, while HER2-specific EXO-T vaccine stimulated HER2-specific immunity against HER2/HLA-A2-expressing BL6-102H2/HER2 melanoma in double transgenic HER2/HLA-A2 mice with HER2-specific self-immune tolerance.22 In addition, HER2-specific EXO-T-stimulated CTLs also showed potent therapeutic effect against both HER2-positive breast cancer T47D and trastuzumab-resistant HER2-positive breast cancer BT474 in athymic nude mice.22 Heterologous DNA vaccines composed of fused cDNA fragments encoding chimeric NH2-terminal human HER2 and COOH-terminal rat Neu sequences have been reported to stimulate stronger antibody responses and protective antitumor immunity than either HER2 or Neu DNA vaccine in transgenic mice with HER2-specific self-immune tolerance.29,30 These findings prompted us to have a hypothesis that heterologous HER2/Neu-specific T cell vaccine may induce more effective anti-HER2 CTL responses. To test this hypothesis, we construct an adenoviral vector (AdVHER2/Neu) expressing a fused cDNA fragment (Hu/Rt HER2/Neu) encoding chimeric NH2-terminal human (Hu) HER2 and COOH-terminal rat (Rt) Neu sequence by recombinant DNA technology.31 Based on AdVHER2/Neu, we further generated heterologous HuRt HER2/Neu-specific EXO-T vaccine (HuRt-Texto) using polyclonal CD4+ T cells with uptake of AdVHER2/Neu-transfected DC-release EXOs.31 We demonstrated that heterologous HuRt-Texto vaccine, in comparison with homologous HER2-Texto one, more strongly stimulated both HER-2-specific antibody and CTL responses leading to complete inhibition of growth of established lung metastasis of HER2-expressing 4T1HER2 breast cancer in BALB/c mice and complete protection of transgenic HLA-A2/HER2 mice from growth of HLA-A2/HER2-expressing BL6-102H2/HER2 melanoma in double transgenic HER2/HLA-A2 mice.31 In addition, HuRt-Texto-stimulated CTLs are also able to
eradicate established trastuzumab-resistant BT474 breast cancer in athymic nude mice.31

The long-term goal is to develop human therapeutic HER2 Neu-specific EXO-T vaccine using autologous polyclonal T cells with uptake of HER2 Neu-specific autologous DC(HER2 Neu)-released EXOs as a new novel personalized vaccine for breast cancer.32 The human autologous DCs derived from peripheral blood monocytes activated in culture medium by granulocyte-macrophage colony-stimulating factor, IL-4, and tumor necrosis factor-α33 followed by infection with HER2 Neu-specific adenoviral vector (AdVHER2 Neu) to form DCHER2 Neu.22

Conclusion

Taken together, our data indicate that HER2-specific EXO-T vaccine circumventing HER2 tolerance may provide a new therapeutic alternative for trastuzumab-resistant breast cancer patients with HER2-specific self-immune tolerance. Because many other human cancer antigens were also identified including α-fetal protein, carcinoembryonic antigen, CA125, CA19-9, and prostate-specific antigen in various types of cancer,34 novel EXO-T vaccines similarly generated by arming polyclonal CD4+ T cells with different tumor antigen-specific EXOs are thus likely to become a useful therapeutic strategy to assist in the treatment of various cancers.

Disclosure

The authors report no conflicts of interest in this work.

References