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REVIEW

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

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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 naïve 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

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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.[13] Apart from those signals, IL-15 secreted by DCs induces T-cell memory formation.[13]

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,[14,15] indicating that HER2 is an immunogenic target for the development

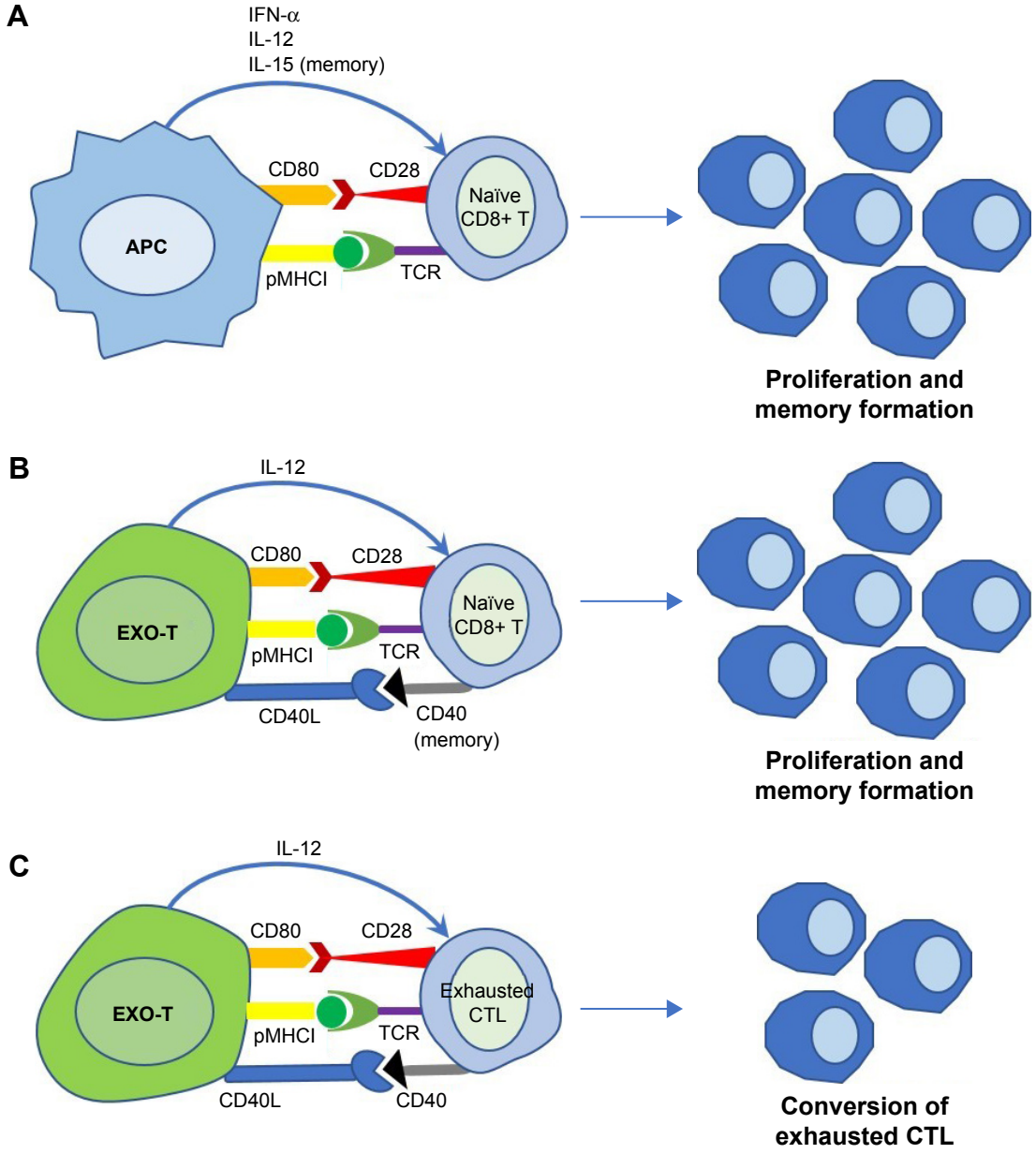

Figure 1 Functional characteristics of the novel EXO-T vaccine.
Notes: (**A**) Conventional three signals in APC-stimulated CD8+ T-cell responses, including 1) antigen peptide/major histocompatibility complex-I (pMHC-I)/TCR, 2) costimulatory CD80/CD28, and 3) cytokines IL-1α, IL-12 (for T-cell functional development), and IL-15 (for T-cell memory formation). (**B**) Distinct three signals derived from novel EXO-T vaccine include 1) exosomal pMHC-I/TCR, 2) exosomal CD80/CD28 and T-cell CD40L/CD40 (for T-cell memory formation), and 3) T-cell cytokine IL-2 (for T-cell proliferation). (**C**) Conversion of exhausted CD8+ CTLs within tumor by EXO-T cells via T cell CD40L/CD40-activated mTORC1 pathway.
Abbreviations: APC, antigen-presenting cell; CTLs, cytotoxic T lymphocytes; EXO, exosome; IFN-γ, interferon-γ; TCR, T-cell receptor.

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 responses[19–25] 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-Texo vaccine

We have recently developed Neu-specific (the rat's form of human HER2) or HER2-specific EXO-T vaccines (Neu-Texo and HER2-Texo) 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 FVBneuN mice, while HER2-specific EXO-T vaccine stimulated HER2-specific immunity against HER2/HLA-A2-expressing BL6-$10_{A2/HER2}$ 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 ($AdV_{HER2/Neu}$) 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 $AdV_{HER2/Neu}$, we further generated heterologous HuRt HER2/Neu-specific EXO-T vaccine (HuRt-Texo) using polyclonal $CD4^+$ T cells with uptake of $AdV_{HER2/Neu}$-transfected DC-release EXOs.[31] We demonstrated that heterologous HuRt-Texo vaccine, in comparison with homologous HER2-Texo 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 $4T1_{HER2}$ breast cancer in BALB/c mice and complete protection of transgenic HLA-A2/HER2 mice from growth of HLA-A2/HER2-expressing BL6-$10_{A2/HER2}$ melanoma in double transgenic HER2/HLA-A2 mice.[31] In addition, HuRt-T_{EXO}-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 ($DC_{HER2/Neu}$)-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-α[33] followed by infection with HER2/Neu-specific adenoviral vector ($AdV_{HER2/Neu}$) to form $DC_{HER2/Neu}$.[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.

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