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LETTER TO THE EDITOR

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A hybrid of B and T lymphoblastic cell line could potentially substitute dendritic cells to efficiently expand out Her-2/neu-specific cytotoxic T lymphocytes from advanced breast cancer patients in vitro

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Abstract

Adoptive transfer of cytotoxic T lymphocytes (CTLs) holds promises to cure cancer. However, this treatment is hindered by lacking a robust way to specifically expand out CTLs. Here, we developed a hybrid of B lymphoblastic cell line and T lymphoblastic cell line (T2 cells) as a substitute of dendritic cells, together with irradiated autologous peripheral blood mononuclear cell (PBMC) as feeder cells and rhIL-2, to activate and expand Her-2/neu-specific $CD8^+$ T cells from human epidermal growth factor receptor 2 (Her-2/neu) and human leukocyte antigen (HLA)-A2 double positive advanced breast cancer patients in vitro. These Her-2/neu-loaded T2 cells reproducibly activated and expanded out Her-2/neu-specific $CD8^+$ T cells to 10^7 in 8 weeks. Furthermore, these Her-2/neu-specific $CD8^+$ T cells had good sensitivity of recognition and killing Her-2/neu-overexpressed breast cancer cell line SK.BR.3. This technique gives us another insight on how to rapidly obtain sufficient CTLs for adoptive cancer immunotherapy.

Keywords: Hybrid of B and T lymphoblastic cell line, Adoptive cancer immunotherapy, Cytotoxic T lymphocytes, Breast cancer

To the editor

The current standard way to expand specific cytotoxic T lymphocytes (CTLs) replies obtaining sufficient dendritic cells (DCs) from patients (Additional file 1). This method has several defects such as invasive, time-consuming, expansive, and unstable according to patients' physical conditions [1–5]. Our group found that the T2 cells, which are a cloned hybrid between the 721.174 (variant of the B lymphoblastic cell line LCL 721) and $CEM^R.3$ (variant of T lymphoblastic cell line CEM-C7), potentially conform to the demands. The cells are TAP and MHC class II deficient, but they do express HLA-A2 and massive co-stimulatory molecules (Additional file 2: Figure S1). These characters make T2 cells potentially useful to study $CD8^+$ T cell recognition of MHC class I antigens, meanwhile, convenient to exclude from MHC class II antigen intervention. Subsequently, HER-2/neu$_{(369–377)}$ and HER-2/neu$_{(435–443)}$ which scores more than 20 by SYFPEITHI prediction was selected to load to T2 cells to expand Her-2/neu-specific $CD8^+$ T cells. HIV gag$_{(77–85)}$, insulin B chain$_{(34–42)}$, and HER-2/neu$_{(39–47)}$ which scores minus 3 were performed as a control (see Additional file 1: Table S1). All peptides except low-affinity peptide HER-2/neu$_{(39–47)}$ could stabilize HLA-A2 molecules on the cell membrane obviously (Additional file 3: Figure S2A).

After co-culture with $CD8^+$ T cells from Her-2/neu and HLA-A2 double positive advanced breast cancer patients, HER-2/neu$_{(369–377)}$- and HER-2/neu$_{(435–443)}$-loaded T2 cells lead to a large secretion of IFN-γ, but not the three controls (Additional file 3: Figure S2B).

For the expansion, starting from 1×10^5 total $CD8^+$ T cells that were less than 0.05% Her-2/neu-specific, nearly

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10^7 HER-2/neu$_{(369–377)}$ or HER-2/neu$_{(435–443)}$-specific CD8$^+$ T cells were expanded out in 8 weeks with the purity of around 85% (Fig. 1a, b). The expanded specific CD8$^+$ T cells got massive co-stimulatory molecules and activation makers (Fig. 1c). The three controls got negative results. That was expected due to extremely low frequency of naturally occurring HIV gag$_{(77–85)}$ and insulin B chain$_{(34–42)}$-specific naive CD8$^+$ T cells as

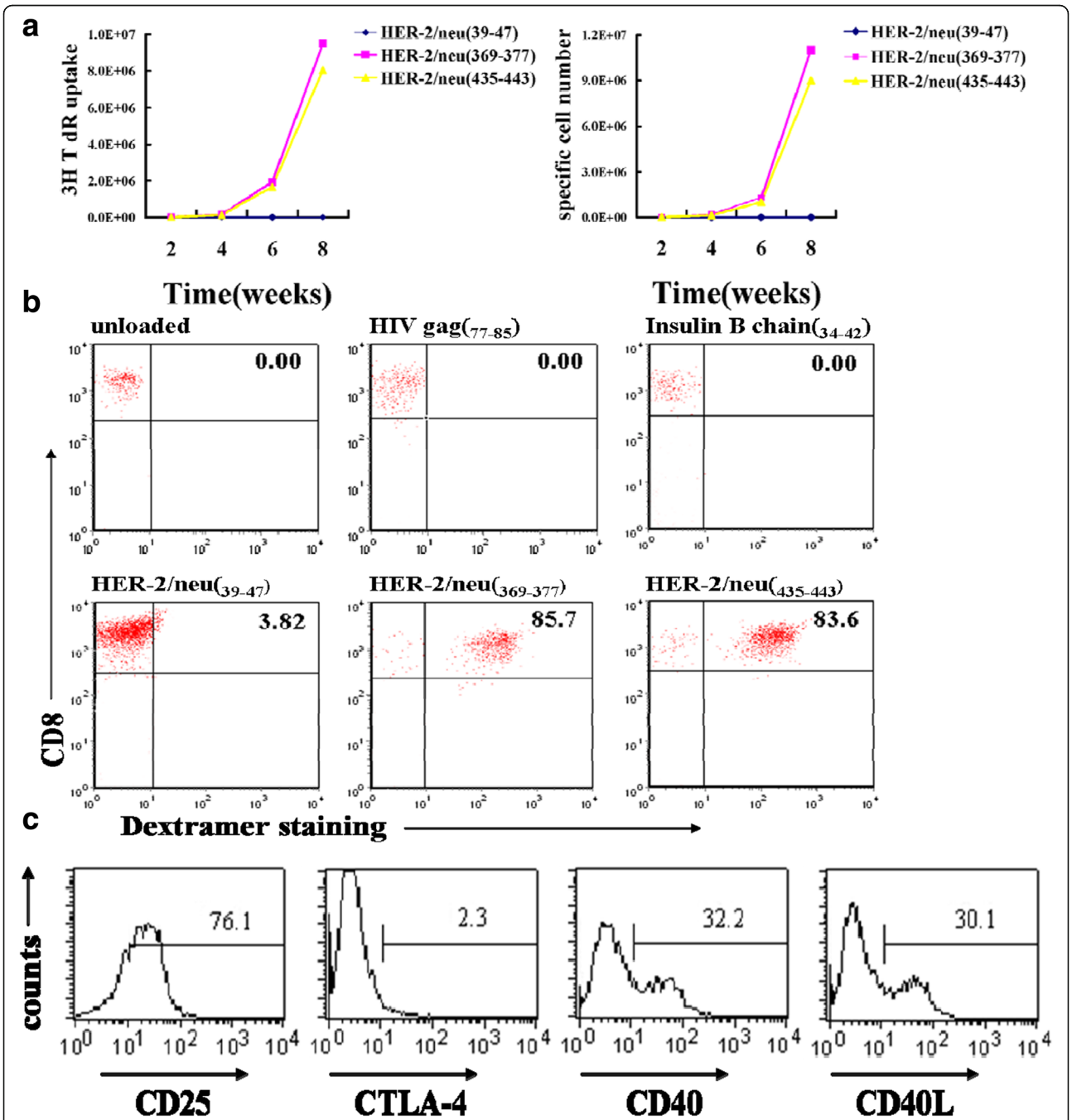

Fig. 1 Her-2/neu-specific CD8$^+$ T cells are efficiently expanded out by Her-2/neu-loaded T2 cells from advanced breast cancer patients. **a** In vitro Her-2/neu-specific CD8$^+$ T cells were expanded out by Her-2/neu-loaded T2 cells in addition with rhIL-2 and irradiated autologous PBMC (peptides, HER-2/neu$_{(39–47)}$, HER-2/neu$_{(369–377)}$, and HER-2/neu$_{(435–443)}$, respectively). *Left*, proliferation assessed as uptake of [^{3}H] thymidine; *right*, total cell number counting of specific CD8$^+$ T cells after expansion. Nearly 1.0E + 07 Her-2/neu-specific CTLs were expanded out in 8 weeks. **b** Her-2/neu-specific CD8$^+$ T cells were verified by Her-2/neu-conjugated HLA-A*0201 Dextramer staining by fluoresence. Her-2/neu$_{(369–377)}$- and Her-2/neu$_{(435–443)}$-loaded T2 cells expanded out specific CD8$^+$ T cells with the purity of around 85%. **c** The expanded Her-2/neu specific CD8$^+$ T cells expressed massive activation makers CD25, CD40, and CD40L. The results are all from one representative of three independent experiments

indicated in Additional file 3: Figure S2B and low affinity HER-2/neu$_{(39–47)}$ peptides as showed in Additional file 1: Table S1. We found these expanded HER-2/neu-specific CD8$^+$ T cells were mainly effector and effector memory cells (Additional file 4: Figure S3).

The expanded Her-2/neu-specific CTL populations mediated dose-dependent lysis to HLA-A2-positive and Her-2/neu-overexpressed breast cancer cell line SK.BR.3, but not to the three control targets (Fig. 2a). The lysis attributed to over 50% perforin producing CTLs and more than 80% granzyme B producing CTLs (Fig. 2b). The cytotoxic activity against SK.BR.3 could significantly be eliminated by W6/32, but not by IgG2 (shown in Fig. 2c).

A major finding of this study was that the Her-2/neu-loaded T2 cells could expand out nearly 10^7 Her-2/neu-specific CTLs in 8 weeks. The expanding efficiency is equivalent to DCs previously reported by Marzocchetti et al. [6–8]. And because the expansion was started from

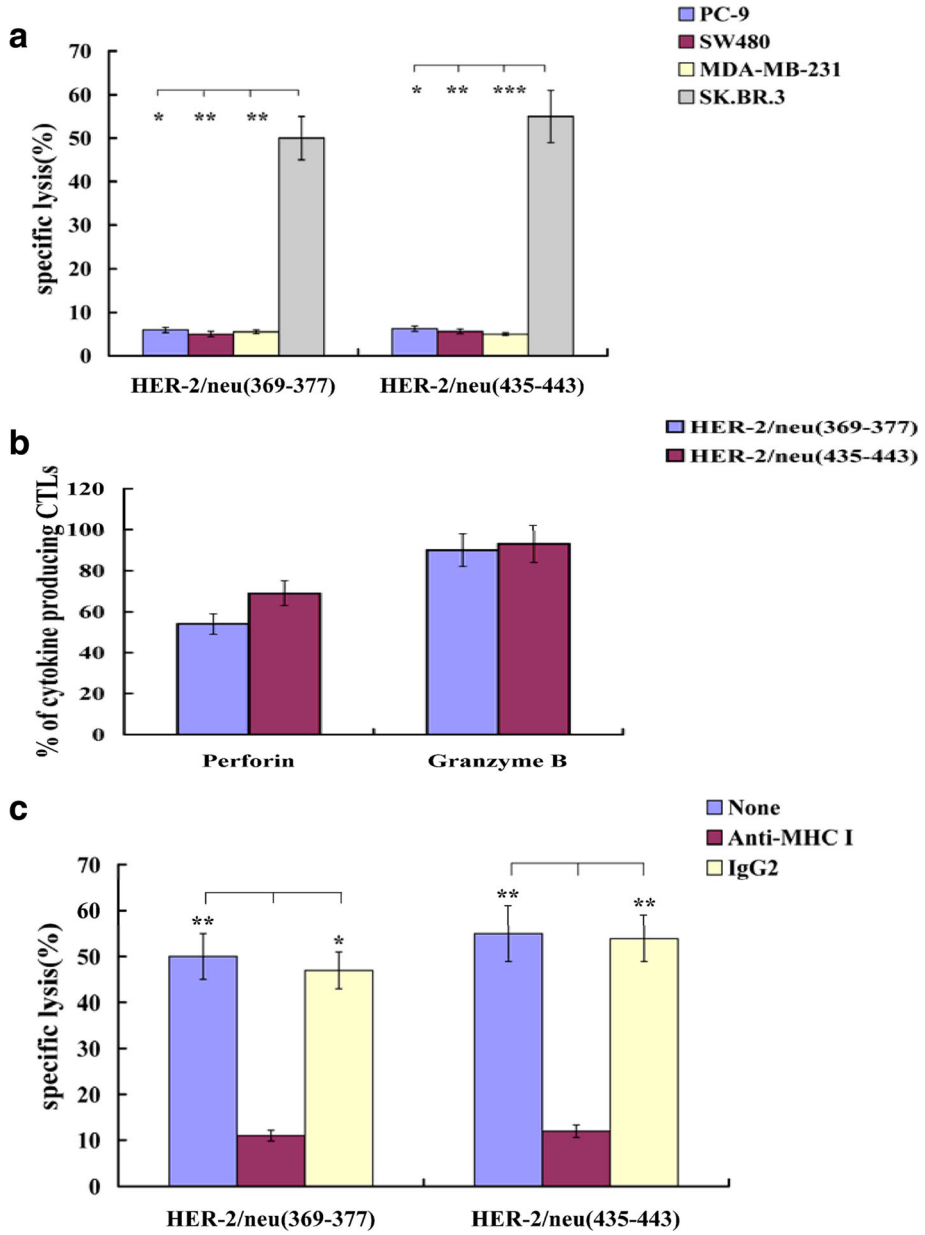

Fig. 2 The expanded Her2/neu-specific CTLs well recognize and kill the related targets. **a** The expanded effector cells (HER-2/neu$_{(369–377)}$-or HER-2/neu$_{(435–443)}$-specific CD8$^+$ T cells) could only lyse HLA-A2-positive and Her-2/neu-overexpressed breast cancer cell line SK.BR.3, but not the controls. **b** Perforin or granzyme B secretion by expanded Her2/neu-specific effector cells was detected after co-culture with SK.BR.3. **c** Anti-HLA class I antibody blocking inhibited the cytotoxic activity of the expanded Her-2/neu-specific CD8$^+$ T cells against SK.BR.3. Mouse IgG2 antibody was added as a control. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ (ANOVA)

$CD8^+$ T cells which were only from 5 ml blood, with an initial frequency of Her-2/neu-specific $CD8^+$ T cells at 0.05%, it would be possible to amplify the expanding quantity if we isolate the Her-2/neu-specific $CD8^+$ T cells firstly from more blood, 50 ml or more for example.

We found the expanded HER-2/neu-specific CTLs could recognize endogenous antigen on allogeneic breast cancer cell line SK.BR.3. This is critically important because previously, many expanding techniques lead to CTLs that could only kill targets pulsed with related peptides but not targets that endogenously processed the antigen of interest [9]. Yee et al. [10, 11] found low affinity of induced CTLs leads to failure of recognition of endogenous antigen.

Thus, T2 cells have shown promise as a convenient tool to rapidly expand out Her-2/neu-specific CTLs in vitro. But so far, we are not able to transfer the expanded Her-2/neu-specific CTLs to breast cancer patients directly as they are mixed with T2 cells, and for the same reason, we cannot compare its anti-tumor effect with trastuzumab in breast cancer patients either. This technique might accelerate to study expanding CTLs in vitro and promote the development of safe and effective adoptive cancer immunotherapy in the future.

Additional files

Additional file 1: Materials and methods. (DOC 199 kb)

Additional file 2: Figure S1. T2 cells express more HLA-A2 and equivalent co-stimulatory molecules compared with DC cells. (TIF 173 kb)

Additional file 3: Figure S2. Her-2/neu-loaded T2 cells can activate related $CD8^+$ T cells equally as DCs. a T2 cells stabilized the MHC I molecules on the cell membrane after loading relative restricted peptides. The mean fluorescence intensity of HLA-A2 was detected by FACS staining before or after peptide loading. b Her-2/neu-loaded T2 cells could activate $CD8^+$ T cells as effectively as DC cells. IFN-γ secretion of $CD8^+$ T cells after activation was detected by ELISA. $^*P < 0.05$, $^{**}P < 0.01$ (Student's *t* test). (TIF 14741 kb)

Additional file 4: Figure S3. The expanded Her-2/neu-specific $CD8^+$ T cells are mainly effector and effector memory cells. CCR7 and CD45RA expression on expanded $CD8^+$ T cells was detected by FACS. The expanded Her-2/neu specific CTLs were partially $CCR7^-CD45RA^+$ (effector), partially $CCR7^-CD45RA^-$ (effector memory), and rarely (about 1.62%) $CCR7^+CD45RA^+$ (naive). This representative data are from the expanded Her-2/$neu_{(369–377)}$-specific $CD8^+$ T cells. (TIF 11635 kb)

Abbreviations

CCR7: C-C chemokine receptor type 7; CD45: Cluster of differentiation 45; CTLs: Cytotoxic T lymphocytes; DCs: Dendritic cells; Her-2/neu: Human epidermal growth factor receptor 2; HIV: Human immunodeficiency virus; HLA: Human leukocyte antigen; IL-2: Interleukin-2; MHC: Major histocompatibility complex; PBMC: Peripheral blood mononuclear cell; TCR: T cell receptor

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Availability of data and materials

The dataset supporting the conclusions of this article is available in the SYFPEITHI repository, http://www.syfpeithi.de/bin/MHCServer.dll/EpitopePrediction.htm.

Authors' contributions

SC, LL, and GW conceived and designed the study. SC wrote the manuscript. KL and FG performed the experiments. FG collected and assembled the data. WG performed the statistical analysis. LL reviewed the manuscript. All authors approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable.

Ethics approval and consent to participate

The study was reviewed and approved in 2010 by the Ethics Committee of Tongji Medical College, Huazhong University of Science and Technology. All subjects were performed in accordance with the medical experiment guidelines of Huazhong University of Science and Technology, which abides by the Helsinki Declaration on ethical principles for medical research involving human subjects. Written informed consent to participate under the ethics, consent, and permissions was obtained from all ten patients.

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