News Release

Title

Introduction of Genetically-Modified CD3ζ Improves Proliferation and Persistence of Antigen-specific CTLs

Key Points

- We newly generated two types of artificial T cell activating adapter molecules (ATAMs), which were CD3ζ/CD28 and CD3ζ/4-1BB.
- Particularly the CD3ζ/4-1BB, ATAM-transduced TCR-CTLs exhibited improved proliferation, persistence and anti-tumor effect.
- ATAMs can be combined with various types of TCR-T therapy.
- Because ATAM-transduction does not change the target recognition site of the TCR, ATAMs transduction would safely improve the effect of TCR-T therapies.

Summary

Graduate student Kotaro Miyao (1st author), Assistant Professor Seitaro Terakura (corresponding author), Professor Hitoshi Kiyoi, and their collaborators in the Department of Hematology and Oncology, Nagoya University Graduate school of medicine (Dean: Kenji Kadomatsu) and Toyama University generated two artificial T cell activating adapter molecules (ATAMs) for improving T cell receptor gene transfer T cell (TCR - T) therapy, one of cancer immunotherapy.

Cancer immunotherapy is becoming a new therapeutic option for malignant tumors, which is mostly difficult to cure with conventional anticancer drug therapy, but the effect of TCR-T therapy has been limited to date. There is also the danger of causing unexpected side effects when TCR-affinity alteration is attempted to improve the effect of TCR-T. Therefore, The researchers developed two types of ATAM, CD3ζ/CD28 and CD3ζ/4-1BB molecules in order to improve the effect of TCR-T without modifying TCR. These are new molecules generated by fusing CD28 and 4-1BB, which are costimulatory molecules of T cells, to CD3ζ molecules.

Introduction of the ATAMs into T cells enhanced the signal to the T cells only when antigenic stimulation is delivered, the proliferative capacity was improved and it could be maintained for a long time. ATAMs transduced T cells improved tumor suppression in mice. The breakthrough points are high safety and versatility because it can be used in combination with various T cell receptors. This study was published online in *Cancer Immunology Research* on April 13, 2018.

Research Background

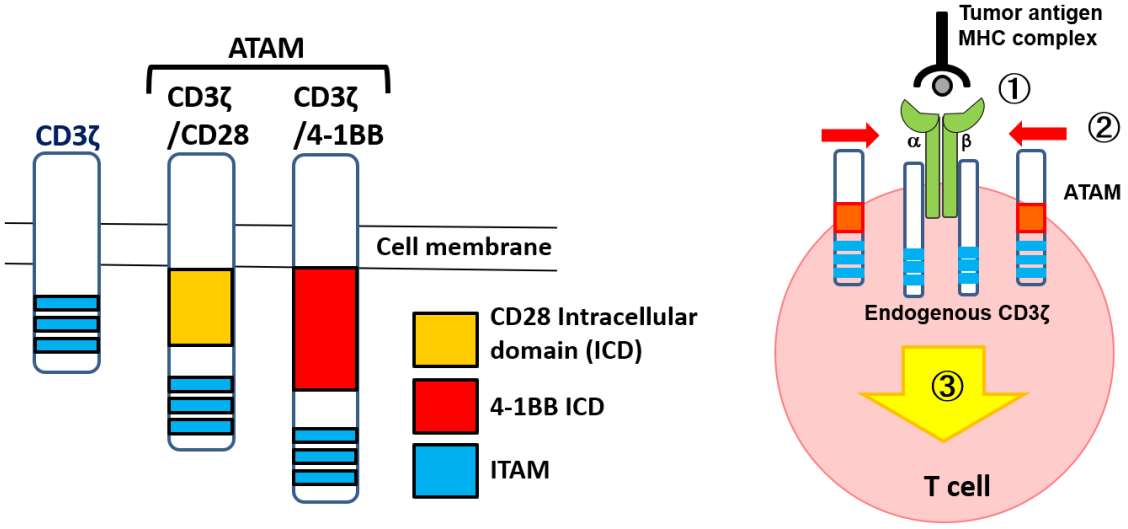
Cancer immunotherapies applying T cell immunity such as immune checkpoint inhibitors, CAR-T therapies, and TCR-T therapies has been put to clinical use one after another. They are

becoming new treatment options for malignant tumors. Although TCR-T therapies targeting to various cancer antigens are undergoing clinical trials, they showed only a short-term effect on limited cases. In order to improve the efficacy, the TCR affinity has been modified. However such TCR modifications caused severe life threatening toxicities such as brain damage or cardiotoxicities. Thus we need to improve the effect of TCR-T therapies safely.

Research Results

To improve the TCR-T therapies without TCR affinity modification, we generated two artificial T-cell activated adapter molecules (ATAMs), which were CD3ζ/CD28 and CD3ζ/4-1BB (Figure 1). They are incorporating costimulatory molecules, CD28 or 4-1BB. When the T cell recognizes the target by TCR α and β chain, CD3ζ-like ATAMs could assemble with the TCR complex and enhance the intracellular signals without altering the antigen specificity. ATAMs were expressed on the cell surface of the introduced cells (Figure 2). When ATAMs were transduced, cytomegalovirus-specific T cells and cancer antigen NY-ESO-1 specific T cells showed better proliferation and persistence after specific antigen stimulation (Figure 3). It was desirable functional change for TCR-T therapies. Subsequently, we examined the changes in the antitumor effect. U266 which is a multiple myeloma cell line expresses cancer antigen NY-ESO-1. Multile myeloma model mice were generated by transplanting U266 into immunocompromised mice. As a treatment, NY-ESO-1 specific T cells were intravenously injected. Nonspecific T cells could not suppress U266 growth in mice. NY-ESO-1 specific T cells decreased U266, in particular CD3ζ/4-1BB transduced NY-ESO-1 specific T cells greatly reduced tumor burden, and the effect lasted for a longer time (Figure 4). These results indicated that CD3ζ/4-1BB improved the therapeutic effect of tumor-specific TCR-T therapies. In a series of experiments, ATAMs-transduced T cells did not activate without specific antigen and the reaction to the specific antigen was not excessive. The safety was confirmed.

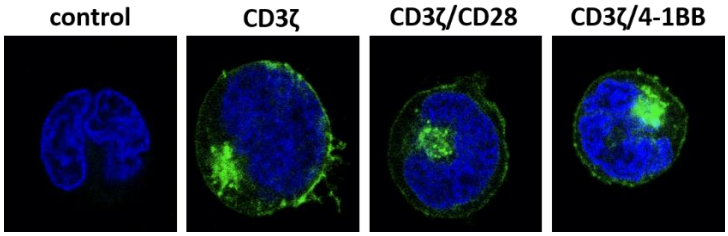
Figure 1. Generation of ATAMs and the hypothesis of T cell functional change.



As shown in left figure, ATAMs have CD3ζ like structures. Right figure shows the strategy of

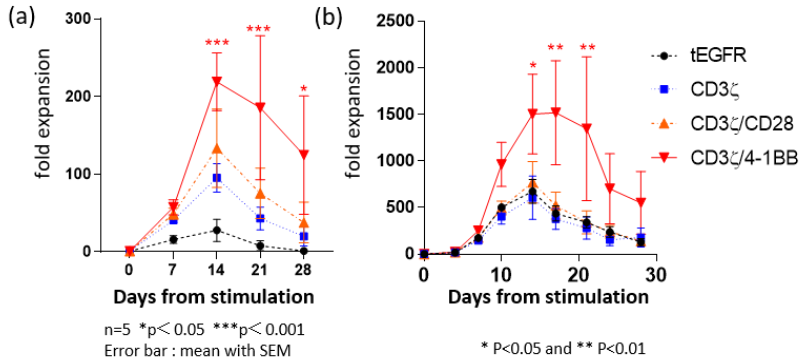
ATAMs transduction effect. When the T cell recognizes the target by TCR α and β chain, CD3ζ-like ATAMs could assemble with the TCR complex and enhance the intracellular signals. As the result, ATAM-transduced T cell could improve its function.

Figure 2. ATAMs expression on live cells



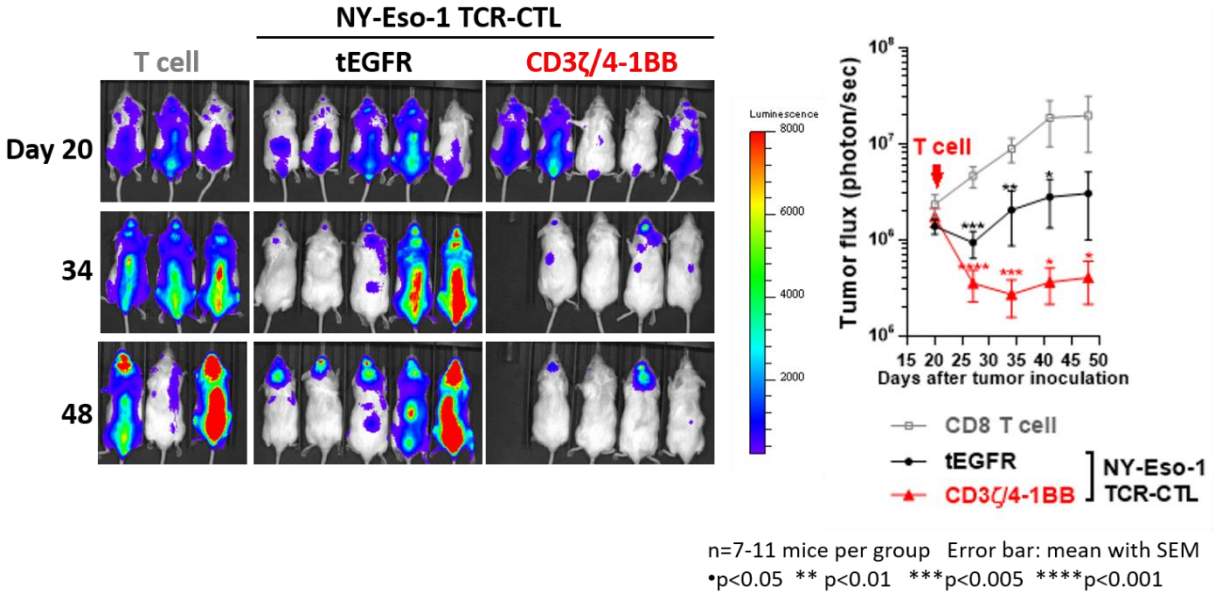
Confocal microscopy images of GFP-fusion ATAM-transduced cell line. ATAMs expressed on the cell surface (green). Nuclei are visualized with DAPI staining (blue).

Figure 3. Improvement of growth of T cells by ATAM transduction.



When ATAMs were transduced, (a) cytomegalovirus-specific T cells and (b) cancer antigen NY-ESO-1 specific T cells showed better proliferation and persistence after specific antigen stimulation comparing with untransduced T cells. Especially, CD3ζ/4-1BB showed greatest improvement. tEGFR was the ATAM untransduced control.

Figure 4. Treatment of multiple myeloma by ATAM-transduced T cells.



Mice luminescence reflects the amount of transplanted U266 cells. T cells were intravenously injected on day 21 after injection of U266 cells into mice. Injection of normal T cells does not decrease U266. NY-ESO-1 specific T cells decreased U266, in particular CD3ζ/4-1BB transduced NY-ESO-1 specific T cells greatly reduced the luminescence, and the effect prolonged for a long time. This data indicate the expansion of anti-tumor effect by CD3ζ/4-1BB transduction.

Research Summary and Future Perspective

ATAM-transduced TCR-CTLs exhibited improved proliferation and persistence. In combination with various tumor-specific TCRs, this strategy of controlling the intracellular signaling of TCR-T cells with ATAM-transduction may be a genetic modification approach to improving the efficacy of TCR-T therapy. We have to prove the efficacy of ATAM-transduction strategy in a clinical trial.

Publication

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Japanese ver.

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