主論文の要旨

Development of TCR-T cell therapy targeting mismatched HLA-DPB1 for relapsed leukemia after allogeneic transplantation

（同種移植後再発白血病に対する不適合ヒト組織適合性抗原DPB1を標的としたT細胞受容体遺伝子改変T細胞の開発）

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【Introduction】

Relapsed hematological malignancies after allogeneic hematopoietic stem cell transplantation (allo-HSCT) remains a significant challenge, with the re-emergence of the primary disease being the major cause of death in approximately 50% of cases. Human leukocyte antigen (HLA) matching between recipient and donor is a critical component of allo-HSCT. Alloreactive donor immune cells can induce an immune response against mismatched HLA antigens in recipient tissues, leading to potentially life-threatening graft-versus-host-disease (GvHD). Therefore, HLA-identical siblings are generally considered optimal options for allo-HSCT. However, such donors are not always available, and allele-level HLA-A, -B, -C, -DRB1, and -DQB1 (10 loci/10 loci)-matched unrelated donors are currently being considered. The alloreactive T cells derived from the donor graft can, on the other hand, recognize and attack residual malignant cells in the recipient's body, causing a graft-vs-leukemia/lymphoma effect (GvL), which is believed to be a good counterbalance of GvHD and responsible for the lower rates of relapse observed in patients who receive allo-HSCT.

GvL-mediated adoptive cell therapy is a form of immunotherapy that involves the infusion of donor-derived immune cells, mainly T cells, into a recipient after allo-HSCT to treat relapsed or refractory hematological malignancies. Donor T cells can be further genetically engineered to express T cell receptors (TCRs) or chimeric antigen receptors (CARs) that target specific tumor antigens to confer the GvL effect. Recent studies have highlighted mismatched HLA-DPB1 as a therapeutic target. Even in 10 loci/10 loci HLA-matched unrelated allo-HSCT, more than 70% of the donor-recipient pairs additionally present mismatches in HLA-DPB1 antigens, and certain HLA-DPB1 mismatches have been shown to correlate with a lower risk of relapse after allo-HSCT. Targeting mismatched HLA-DPB1 antigens carries a low potential risk of GvHD if performed under appropriate conditions because HLA-DP expression is restricted to hematopoietic cells and inflamed non-hematopoietic tissues to a lesser extent. Furthermore, post-allo-HSCT, donor-derived normal hematopoietic cells are also spared because they are 'self' for T cells targeting mismatched recipient-type HLA-DPB1 antigens.

【Research methods】

In this study, we sought to establish T cell clones specific for mismatched HLA-DPB1 antigens using post-HSCT donor-derived CD4+ T cells physiologically primed to mismatched HLA-DPB1 after transplantation. We obtained blood samples from three patients (Allo-001, -004, and -009) diagnosed with hematological malignancies before (pre-) and after (post-) allo-HSCT from HLA-DPB1 mismatched unrelated donors. We optimized the best protocol to induce mismatched HLA-DPB1 specific T cells clones by comparing the stimulation efficiency of CD4+ T cells isolated from 30 and 100 days post-allo-HSCT patients' PBMCs with different stimulators such as HLA-DPB1 mismatched pre-allo-HSCT (recipient origin) PBMCs, donor B-lymphoid cell line (LCL) or leukemia cell line K562 transduced with recipient-specific allo-

HLA-DP α and β cDNA along with CD86. Allo-HLA-DP-specific response was tested at the single-cell level by intracellular cytokine staining (ICS) for interferon (IFN)-γ. For enrichment of allo-HLA-DP-specific T cells, an IFN-γ secretion assay followed by MACS sorting was performed. The sorted cells were then cloned by limiting dilution. Growing clones were tested for reactivity against non-hematopoietic HeLa cell line transduced with recipient's HLA-DP α and β cDNA together with HLA-DM and invariant chain by IFN-γ ELISA. Clones that showed no reactivity against HeLa transfectants were selected for further detailed analysis.

Putative clones were tested for IFN-γ production against various leukemia cell lines and primary leukemia blasts by ELISA. The potential cross-reactivity of putative clones against a panel of U937 Leukemia cells transduced with various HLA-DP alleles commonly found in the Japanese population was tested by IFN-γ ELISA.

For generation of genetically modified T cells, TCR-α and TCR-β gene sequences from selected T cell clones were determined by RT-PCR and direct sequencing. The TCRα and TCRβ genes were cloned into a retroviral vector and used to infect 5-day CD3/CD28-activated T cells isolated from healthy donors. The resultant TCR-T cells were subjected to functional and specificity analysis.

【Results and Discussion】

From three patients recruited in this study, we isolated multiple T cell clones specific for HLA-DPB1*02:01, -B1*04:02, and -B1*09:01. We prioritized the HLA-DPB1*09:01-restricted clone 2A9 because HLA-DPB1*09:01 is unique and its frequency is relatively high in the Japanese population.

Detailed analysis confirmed clone's 2A9 reactivity against 5/5 of leukemia cell lines in HLA-DPB1*0901 restriction manner. Furthermore, clone 2A9 demonstrated reactivity against primary myeloid leukemia blasts isolated from patients with leukemia, even those with low HLA-DP expression, suggesting that clone 2A9 possess a high-affinity TCR and is promising for clinical application.

TCR-T cells expressing 2A9 TCR retained the ability to trigger HLA-DPB1*09:01-restricted recognition and lysis of various leukemia cell lines and showed multifunctionality# indicated by multiples cytokines production *in vitro*. Notably, not only CD4+ fractions but also CD4-negative fractions similarly produced IFN-γ, suggesting CD8+ TCR-T cells expressing high affinity 2A9 TCR do not require CD4 accessory protein to stably ligate to HLA-DPB1*09:01-expressing cells.

Currently, analysis of TCR repertoire of other putative clones specific to other HLA-DPB1 alleles than HLA-DPB1*09:01 and preparation of TCR-T cells is ongoing.

【Conclusion】

In this study we proved that induction of mismatched HLA-DPB1-specific T cell lines and

clones from alloreactive post-allo-HSCT CD4+ T cells was feasible. Furthermore, we demonstrated that redirection of T cells with a cloned TCR repertoire was readily possible without increased risk of cross-reactivity. Like other single-antigen targeting therapies (e.g., chimeric antigen receptor T cells), one potential limitation of our approach is evasion of antigen-loss variant tumors at genetic level. However, most relapse in HLA class II-mismatched HSCT is reported to be due to epigenetic changes leading to downregulation of class-II antigens which may be reversible. For future clinical applications, further efforts to generate TCR panels that cover common HLA-DP alleles, establish *in vivo* therapeutic models, and development of treatment to restore HLA class II expression are necessary.