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LETTER TO THE EDITOR

Feasibility of gene-immunotherapy using WT1-specific T-cell receptor gene transfer for infant acute lymphoblastic leukemia with *MLL* gene rearrangement

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Infant acute lymphoblastic leukemia (ALL) with rearrangement of the *mixed-lineage leukemia* (*MLL*) gene shows a poor outcome, despite intensive chemotherapy followed by hematopoietic stem cell transplantation at first remission.[1] Furthermore, therapy-related late effects, including short stature and neuroendocrinological complications, remain problematic.[2] This current situation has prompted us to establish a novel treatment for infant ALL based on a new concept. In the present study, we verified the feasibility of adoptive immunotherapy for *MLL* gene-rearranged infant ALL using T lymphocytes genetically engineered by WT1-specific *T-cell receptor* (*TCR*) gene transfer.

Construction of a novel retrovirus vector expressing HLA-A*24:02-restricted WT1-specific TCR with silencing of the endogenous *TCR* gene has been reported previously.[3] The *TCR-α* and *TCR-β chain* genes were cloned from an HLA-A*24:02-restricted $WT1_{235-243}$-specific $CD8^+$ cytotoxic T lymphocyte (CTL) clone, TAK-1.[4] Retrovirus transduction into $CD8^+$ T lymphocytes was performed as described previously.[3] Briefly, $CD8^+$ T lymphocytes were isolated from peripheral blood mononuclear cells of healthy individuals using $CD8^+$ cell-isolating immunomagnetic beads (MACS beads; Miltenyi Biotec, Auburn, CA, USA). $CD8^+$ T lymphocytes were then cultured in RPMI1640 medium containing 10% human serum, anti-CD3 monoclonal antibody (OKT3), interleukins (IL)-2, IL-7, IL-15 and IL-21 for 3 days. The $CD8^+$ T lymphocytes were then transduced with retrovirus vector using the RetroNectin-bound virus infection method in which retroviral solutions were preloaded onto RetroNectin (Takara Bio, Shiga, Japan)-coated plates, and centrifuged at $2000 \times g$ for 2 h. Retrovirus vector-transduced $CD8^+$ T lymphocytes were then cultured in the medium described above for a further 10–14 days. Using this method, more than 60% of $CD8^+$ T lymphocytes appeared to be positive for $WT1_{235-243}$ peptide/HLA-A*24:02 tetramer staining. We used these cells as WT1-specific *TCR* gene-transduced effector cells, that is, WT1-TCR CTLs, for further experiments.

We first examined WT1 expression in infant ALL cell samples obtained from bone marrow of the patients using quantitative real-time polymerase chain reaction of WT1 mRNA and glyceraldehyde-3-phosphate dehydrogenase mRNA as an internal control, as reported previously.[5] Approval for this study was obtained from the institutional review boards of Ehime University Hospital and hospitals registered by the Japan Pediatric Leukemia-Lymphoma Study Group. Written informed consent was obtained from all patients and healthy volunteers in accordance with the Declaration of Helsinki. As shown in Figure 1a, expression of WT1 mRNA appeared to be significantly higher in leukemia cells of ALL infants with *MLL* gene rearrangement ($n=41$) than in those of ALL patients with germline configuration ($n=19$) (*t*-test; $P<0.01$). The expression of WT1 mRNA was extremely low or undetectable in normal peripheral blood mononuclear cells. We also examined the expression of WT1 protein in leukemia cell lines and normal peripheral blood mononuclear cells. As shown in Figure 1b, WT1 protein appeared to be expressed abundantly in all ALL cell lines with *MLL* rearrangement and K562 cells, but not in normal peripheral blood mononuclear cells.

Next, we examined the cytotoxicity of WT1-TCR CTLs against various leukemia cell lines using standard ^{51}Cr-release assays. The results are shown in Figure 2a. WT1-TCR CTLs exerted cytotoxicity against the HLA-A*24:02-positive ALL cell lines with MLL rearrangement (KOCL69, KOPB26, and KOCL44) but not against the HLA-A*24:02-negative ALL cell lines with *MLL* rearrangement (KOCL45 and KOCL51). The K562 cell line, which is negative for HLA expression and sensitive to natural killer cell-mediated cytotoxicity, also appeared to be resistant to the cytotoxicity mediated by WT1-TCR CTLs. As shown in Figure 2b, the cytotoxicity mediated by WT1-TCR CTLs against ALL cell lines with *MLL* rearrangement was significantly inhibited by addition of anti-HLA class I framework monoclonal antibody but not by anti-HLA-DR monoclonal antibody. These results show that WT1-TCR CTLs can exert cytotoxicity against ALL cell lines with *MLL* rearrangement in an HLA-A*24:02-restricted manner through recognition of the $WT1_{235-243}$ epitope that is naturally processed from WT1 protein in ALL cells and presented on the cell surface in the context of HLA class I molecules.

Finally, we examined whether WT1-TCR CTLs can lyse infant ALL cells with *MLL* rearrangement freshly isolated from the patients. As expected, all HLA-A*24:02-positive leukemia cells were lysed by WT1-TCR CTLs; however, HLA-A*24:02-negative leukemia cells were resistant to WT1-TCR CTL-mediated cytotoxicity (Figure 2c). As we have reported previously,[4] WT1-specific CTLs did not exert cytotoxicity against normal cells (data not shown). Taken together, the data suggested that WT1-TCR CTLs appeared to be capable of discriminating infant ALL leukemia cells from normal cells in an HLA-restricted manner.

In the present study, we demonstrated that adoptive immunotherapy for chemotherapy-resistant infant ALL with *MLL* rearrangement using T lymphocytes, genetically engineered by WT1-specific *TCR* gene transfer is feasible, based on the following findings. First, WT1 appeared to be abundantly expressed in most cases of infant ALL, especially those with *MLL* rearrangement, which is the most frequent chemotherapy-resistant infant leukemia. Second, T lymphocytes, genetically engineered by WT1-specific *TCR* gene transfer, efficiently lysed leukemia cells isolated from infants with ALL as well as cell lines with *MLL* rearrangement, but not normal cells, in an HLA-restricted manner.

WT1 is known to have an essential role in development of the kidney and genitourinary system in fetuses. On the other hand, WT1 expression after birth is limited to very few tissues, and its expression level in normal tissues is extremely low. In contrast to the low expression level of WT1 in normal cells, WT1 is expressed abundantly in various kinds of acute leukemia.[6] In addition, WT1 is reportedly expressed in chemotherapy-resistant leukemia stem cells.[7] Using *in vitro* and *in vivo* experimental systems, we and other groups have previously revealed that WT1-specific CTLs never induce cell and tissue damage.[4,8] These findings indicate that cell-mediated immunotherapy for

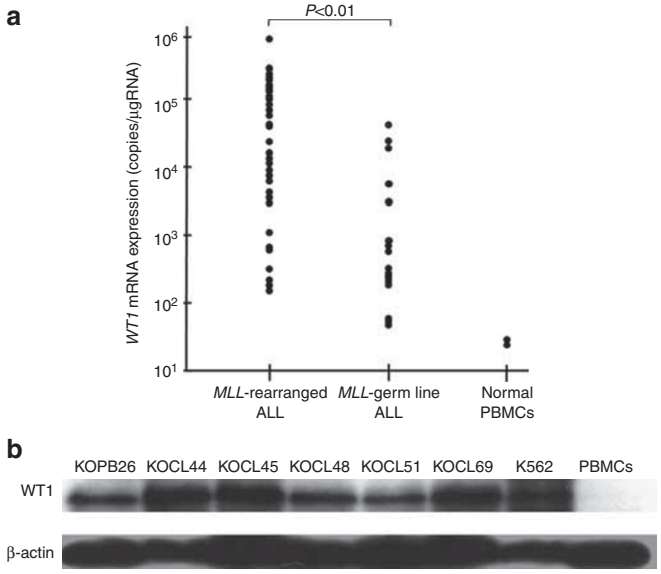

Figure 1 WT1 expression in leukemia and normal cells. (**a**) WT1 mRNA expression in infant ALL cells with or without *MLL* rearrangement and normal peripheral blood mononuclear cells. Expression levels of WT1 mRNA in bone marrow cells of patients with infant leukemia and peripheral blood mononuclear cells of healthy individuals were examined by quantitative real-time polymerase chain reaction. The level of WT1 mRNA expression in infant ALL cases with *MLL* rearrangement was significantly higher than in those with germ-line *MLL* gene ($P<0.01$). (**b**) Expression of WT1 protein in leukemia cell lines and normal peripheral blood mononuclear cells by western blotting. WT1 protein was abundantly expressed in infant ALL cell lines with *MLL* rearrangement, and also in K562 cells.

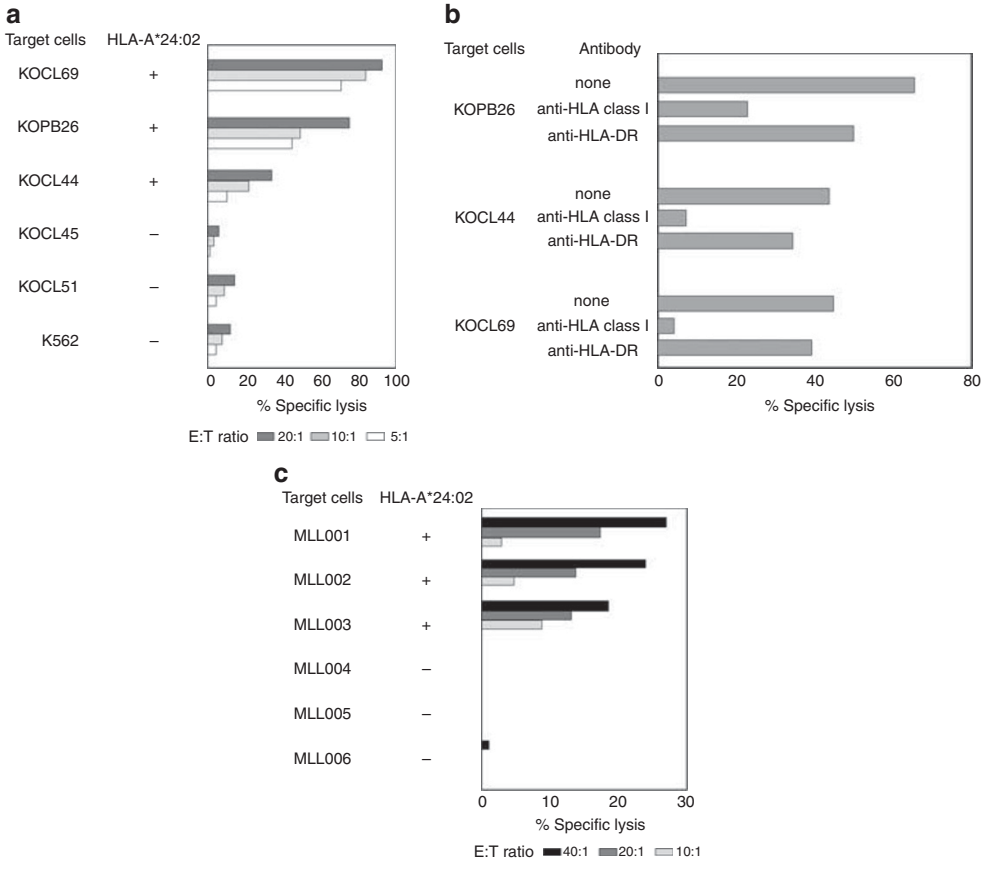

Figure 2 Cytotoxicity of WT1-TCR CTLs against infant ALL cells with *MLL* rearrangement. (**a**) Cytotoxicity of WT1-TCR CTLs against infant ALL cell lines. The cytotoxicity of WT1-TCR CTLs against HLA-A*24:02-positive and HLA-A*24:02-negative infant ALL cell lines with MLL rearrangement, and also the K562 cell line, was determined by 5-h ^{51}Cr-release assays at effector/target ratios of 20:1, 10:1 and 5:1. (**b**) HLA class I restriction of cytotoxicity mediated by WT1-TCR CTLs against infant ALL cells. The cytotoxicity of WT1-TCR CTLs against infant ALL cell lines with *MLL* rearrangement was determined by 5-h ^{51}Cr-release assays at an effector/target ratio of 5:1 in the presence or absence of anti-HLA class I framework monoclonal antibody or anti-HLA-DR framework monoclonal antibody. (**c**) Cytotoxicity of WT1-TCR CTLs against freshly isolated infant ALL cells with *MLL* rearrangement freshly isolated from the patients, as determined by 5-h ^{51}Cr-release assays at effector/target ratios of 40:1, 20:1 and 10:1.

malignancies targeting WT1 is effective and safe. In view of the fact that infant ALL is chemotherapy-resistant and that the indications for hematopoietic stem cell transplantation are limited, in addition to the high prevalence of severe therapy-related adverse events after hematopoietic stem cell transplantation, the clinical efficacy of gene-immunotherapy using WT1-specific *TCR* gene transfer would be considerably advantageous.

Conflict of interest

The authors declare no conflict of interest.

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