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Cancer Immunotherapy Targeting Wilms’ Tumor Gene WT1 Product

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The Wilms’ tumor gene WT1 was first reported as the gene responsible for Wilms’ tumor, a pediatric renal cancer (1, 2). This gene encodes a zinc finger transcription factor involved in tissue development, in cell proliferation and differentiation, and in apoptosis, and is categorized as a tumor suppressor gene (3). The WT1 gene product regulates the expression of various genes either positively or negatively depending upon how it combines with other regulatory proteins in different types of cells.

Tumor Ags can be categorized into five groups: ubiquitous proteins such as mutated ras (4) or p53 (5); tumor-specific shared Ags such as P1A in mice (6) and MAGE in humans (7, 8); differentiation Ags with a good example of tyrosinase (9); overexpressed tumor Ags such as HER-2/neu (10); and Ags derived from oncoviruses with the best example of the E7 oncoprotein of human papilloma virus 11 (11).

We (12, 13) and others (14–17) have identified high expression levels of the wild-type WT1 gene in leukemic cells regardless of the type of disease to clarify the essential role of the WT1 gene in leukemogenesis. On the basis of accumulated evidence (13, 18–20), we have proposed that the wild-type WT1 gene performs an oncogenic rather than a tumor suppressor gene function in hematopoietic progenitor cells. Moreover, we found that among 34 solid tumor cell lines examined, 28 (82%), including lung, gastric, colon, and breast cancer cell lines, expressed the wild-type WT1 gene (21). Cancer cells of lung cancer patients also expressed the WT gene at high levels (21). Growth of WT1-overexpressing tumor cells was specifically inhibited by WT1 antisense oligodeoxynucleotides, thus suggesting a close relationship between WT1 overexpression and tumorigenesis. These results indicate that the WT1 gene product could be a promising tumor-specific Ag belonging to the fourth category of tumor Ags not only for leukemia but also for various types of solid tumors including lung cancers.

It is well known that tumor-specific CD8+ CTLs constitute the most important effectors for antitumor responses and recognize peptides derived from endogenous proteins presented on the cell surface in association with MHC class I molecules (22). It had been first demonstrated that patients who develop significant graft-versus-host disease (GVHD; grade ≥ 2) after allogeneic bone marrow transplantation (BMT) have a significantly lower rate of relapse than patients with either no GVHD or grade 1 GVHD (23). The demonstration of such a graft-vs-leukemia effect after allogeneic BMT provides the most convincing clinical evidence for the effectiveness of tumor immunity.

Peptides that bind to a given MHC class I molecule have been shown to share common amino acid motifs (24). We (25) and others (26) have previously developed a peptide library-based method for predicting MHC class I-binding peptides. MHC-binding scores can be calculated for all of the peptides of eight or nine amino acids in a given protein sequence by using the experimentally obtained specificity profiles of MHC class I molecules. With few exceptions, the binding scores of naturally occurring MHC class I-binding epitopes for CTLs are as high as twice the SD from the mean score of all of the peptides present in the parental proteins.

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Abbreviations used in this paper: GVHD, graft-versus-host disease; BMT, bone marrow transplantation.
The present study shows that WT1-derived peptides, which were predicted to bind to H-2Db molecules according to the peptide library-based scoring system of MHC class I-binding peptides, actually bind to H-2Db molecules. Furthermore, one of the WT1 peptides induced peptide-specific CTLs as a result of in vivo immunization with the peptide of mice, which then rejected the challenges by WT1-expressing tumor cells.

Materials and Methods

Synthesis of peptides

Peptides were synthesized manually or with an ABH430A peptide synthesizer (Applied Biosystems, Foster City, CA) using Fmoc chemistry. They were then purified by RP-HPLC with a C18 Microbondsphere column (Waters Japan, Osaka, Japan). Synthesis of the correct peptides was confirmed with the aid of an API III triple quadrupole mass spectrometer (Sciex, Thornhill, Toronto, Canada), and concentrations of the peptides were determined by means of a MicroBCA assay (Pierce, Rockford, IL) using BSA as the standard. Some peptides were also custom synthesized (Sawady Technology, Tokyo, Japan).

Cells and Abs

FBL3 is a Friend leukemia virus-induced erythroleukemia cell line originated from C57BL/6 (H-2b), and was generously provided by Dr. B. Chesebro (National Institutes of Health, Bethesda, MD) via Dr. M. Miyazawa (Kinki University, Japan). C1498 and EL4 are a WT1–nonexpressing leukemia or lymphoma cell line of C57BL/6 origin, respectively, and was obtained from American Type Culture Collection (ATCC, Rockville, MD). RMA is a Rauscher leukemia virus-induced lymphoma cell line, and RMAS is a TAP-deficient subline of RMA (27). These cell lines were kindly provided by Dr. K. Kärre (Karolinska Institute, Sweden) through Dr. H.-G. Ramnäess (University of Tübingen, Germany) and maintained in DMEM containing 5% FBS. P815 is a mastocytoma originated from DBA/2 mice (ATCC). YAC-1 cells that were used as target cells for NK activity were obtained from ATCC. Murine WT1-expressing C1498-mWT1 and EL4-mWT1 were established, thus indicating the utility of binding scores for finding molecules, and five peptides with comparatively high binding-affinity scores for H-2Db molecules were identified (Table I). All five of these WT1 peptides with higher binding scores also exhibited a relatively higher binding affinity for H-2Db molecules, and some of these WT1 peptides with higher binding scores also exhibited a relatively higher binding affinity for H-2Db molecules, and some WT1-expressing tumor cells were pulsed with the WT1 peptides and then irradiated with 3000 rad.

Peptides was repeated three times at weekly intervals. After 1 wk from the immunization with LPS-activated spleen cells pulsed with the WT1 peptides and then irradiated with 3000 rad. The cells were then washed and stained with FITC-labeled B22.249 mAb and analyzed by FACScan (Becton Dickinson, Mountain View, CA).

Measurement of binding affinity of WT1 peptides for H-2Db molecules

Binding of WT1 peptides to MHC class I H-2Db molecules was measured by a stabilization assay using RMAS cells as described previously (29). Briefly, RMAS cells were incubated at 26°C overnight to accumulate peptide-receptive MHC class I molecules on the cell surface and then mixed with varying concentrations of WT1 peptides in 100 μl of DMEM containing 0.25% (v/v) BSA. After incubation for 30 min at room temperature, the temperature was raised to 37°C and the incubation was continued for 1 h. The cells were then washed and stained with FITC-labeled B22.249 mAb and analyzed by FACScan (Becton Dickinson, Mountain View, CA). The relative binding affinity in μM of OVAII (30) at 37°C for 2 h. After three weekly immunizations, 3 × 10^4 FBL3 leukemia cells were i.p. inoculated into 4–6-wk-old C57BL/6 mice.

Histology

The main organs, including kidney and lung, were removed from the surviving Db126-immunized mice that had rejected the tumor challenges and fixed in Bouin’s solution. Paraffin sections of 8-μm thickness were stained with hematoxylin and eosin by means of standard methods.

Results

Identification of H-2Db-binding peptides

Most CTL epitope peptides can be predicted by means of a peptide library-based scoring system for MHC class I-binding peptides (25, 26). Amino acid sequences of the murine WT1 protein were scanned for peptides with a potential binding capacity for H-2Db molecules, and five peptides with comparatively high binding scores for H-2Db molecules were identified (Table I). Five of these WT1 peptides with higher binding scores also exhibited a relatively higher binding affinity for H-2Db molecules, and some correlation between binding scores and binding affinity (Kd) was established, thus indicating the utility of binding scores for finding peptides which bind to MHC class I molecules. Db126 peptide demonstrated the same order of binding affinity as that of viral Ags (24), which is the strongest Ags for CTL induction. Three peptides (Db126, Db221, and Db235) with anchor motifs for binding to H-2Db molecules (24) were actually used for in vivo immunization.

Induction of CTLs against WT1 peptides

Whether specific CTLs against these three WT1 peptides could be induced by in vivo immunization with these peptides was examined (Fig. 1). Mice were immunized with LPS-activated spleen...
cells pulsed with the peptides. The spleen cells of the immunized mice were then assayed for cytotoxic activity against peptide-pulsed RMAS target cells. WT1 peptide-specific CTLs were induced by immunization with the Db126 peptide, whereas no CTLs were induced by immunization with the Db221 or Db235 peptide. Thus, only the Db126 peptide with the highest binding affinity for H-2D<sup>b</sup> molecules could elicit CTL responses. Therefore, subsequent investigation focused on the CTLs against the Db126 peptide.

To confirm that the Db126 peptide-induced CTLs specifically recognize the Db126 peptide to kill the target cells, three different CTL lines against the Db126 peptide were assayed for cytotoxic activity against RMAS target cells pulsed with increasing concentrations of the Db126 peptide (Fig. 2). Their cytotoxic activity was found to increase in parallel with an increase in peptide concentrations and to reach a plateau. Half the maximal lysis was observed in the range of nanomolar of the peptide. Lysis by the CTL lines of the RMAS target cells pulsed with naturally occurring H-2D<sup>b</sup>-binding peptide influenza A34 NP (Table I) was not observed (data not shown). These results proved that the cytotoxic activity of the CTL lines was specific for the Db126 peptide.

Lysis of endogenously WT1-expressing tumor cells by Db126-specific CTLs

We next investigated whether Db126-specific CTLs could recognize and lyse endogenously WT1-expressing tumor cells. As shown in Fig. 3, a panel of tumor cell lines was tested for lysis by Db126-specific CTL lines. Before the tests, specificity of the CTLs for the Db126 peptide was confirmed by specific lysis of Db126 peptide-pulsed RMAS target cells (Fig. 3a). The Db126-specific

Table I. Binding of WT1 peptides to H-2D<sup>b</sup> molecules

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Amino Acid Sequence</th>
<th>Binding Score</th>
<th>K&lt;sub&gt;a&lt;/sub&gt; (M)</th>
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<tbody>
<tr>
<td>WT1 peptides</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Db126</td>
<td>aa 126–134 RMFPNAPYL</td>
<td>1.77</td>
<td>5.7 × 10&lt;sup&gt;-7&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>aa 227–235 YQMTSQLEC</td>
<td>1.93</td>
<td>1.0 × 10&lt;sup&gt;-6&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>aa 235–243 CMTWQMNGL</td>
<td>1.20</td>
<td>1.3 × 10&lt;sup&gt;-6&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>aa 221–229 YSSDNLYQM</td>
<td>1.05</td>
<td>2.6 × 10&lt;sup&gt;-6&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>aa 136–144 SCLESQPTI</td>
<td>1.61</td>
<td>3.7 × 10&lt;sup&gt;-6&lt;/sup&gt;</td>
</tr>
<tr>
<td>Known epitope peptides</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SV40 T antigen</td>
<td>aa 223–231 CKGVNKEYL</td>
<td>1.85</td>
<td>1.9 × 10&lt;sup&gt;-7&lt;/sup&gt;</td>
</tr>
<tr>
<td>Influenza A34 NP</td>
<td>aa 366–374 ASNENMETM</td>
<td>1.24</td>
<td>1.9 × 10&lt;sup&gt;-7&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

* Bold letters represent anchor motifs.
CTLs lysed endogenously WT1-expressing FBL3 cells, but not WT1-nonexpressing RMA cells (Fig. 3b). Furthermore, the Db126-specific CTLs killed murine WT1-transfected C1498 cells to a significant extent when compared with parental WT1-nonexpressing C1498 cells, confirming that the molecule targeted for killing by the CTLs is indeed the WT1 peptide (Fig. 3c). Similarly, a specific lysis of WT1-nonexpressing EL-4 cells used here (H-2Db) was obtained as a result of transfection of the WT1 gene, although the lysis was weak because of low expression of H-2Dβ molecules on the EL-4 cells used here (Fig. 3d). WT1-nonexpressing P815 cells with nonidentical H-2 molecules were not lysed by the CTLs (data not shown). RMAS cells that were common targets for lymphokine-activated killer/NK cells (31) were not killed by the CTLs (Fig. 3a). However, when YAC-1 cells were used as target cells for NK activity, the Db126-specific CTLs also lysed the cells. This phenomenon is reasonable, since it has been well known that CTLs frequently display an NK-like cytolytic activity in addition to Ag-specific cytolytic activity (32–34). The Db126-specific CTLs were also 99% positive for CD8 and virtually negative for NK1.1 (data not shown). Since various NK-activating/inhibitory receptors are expressed not only on NK cells but also on CTLs bearing TCRαβ (31, 35, 36), these receptors may be responsible for the lysis of YAC-1 cells. Taken together, these results suggest that the Db126-specific CTLs can recognize Db126 or related peptides which are naturally produced through intracellular processing of the WT1 protein and are present on H-2Db molecules in WT1-expressing cells.

Cytotoxic activity is H-2Dβ restricted

Furthermore, to demonstrate that the cytotoxic activity of the CTLs is MHC restricted, it was assayed in the presence of Abs against H-2 class I molecules (Fig. 4). The suppression of cytotoxic activity of the CTLs against WT1-expressing FBL3 cells was found to depend upon an increase in the concentration of Abs against H-2Dβ, but Abs against H-2Kβ or H-2Ld did not show any suppressive effect on the cytotoxic activity of the CTLs. These results showed that the CTLs exert their cytotoxic activity in an H-2Dβ-restricted fashion.

Presence of sensitizing activity to Db126-specific CTLs in cell extract of WT1-expressing cells

To confirm that endogenously WT1-expressing tumor cells express the Db126 peptide on their cell surface through intracellular processing of the WT1 protein and that Db126-specific CTL lines recognize this peptide for cell lysis, WT1-expressing FBL3 cells were lysed and peptide fractions were prepared (Fig. 5). The peptide fractions were further fractionated by RP-HPLC, and each fraction was assayed for its sensitizing activity to the Db126-specific CTLs. The sensitizing activity was recovered in the same retention time as that needed for the synthetic Db126 peptide. One additional sensitizing activity peak was detected. Such additional peaks have previously been observed by us and others when naturally processed endogenous peptides were prepared from whole-cell lysate. In such cases, some peaks represented the sensitizing activity of longer peptides harboring the minimal epitope peptide (37, 38), a tissue-specific variant peptide (39), or cross-recognized peptides bearing similar or unrelated amino acid sequences (40, 41). These results showed that the Db126 peptide targeted by the
Db126-specific CTLs is naturally produced by intracellular processing of the WT1 protein in WT1-expressing cells.

**Eradication of tumor challenges by preimmunization with the Db126 peptide**

We next investigated whether active immunization with the Db126 peptide elicited in vivo tumor immunity. Mice were immunized once a week for 3 wk with LPS-activated spleen cells pulsed with the Db126 peptide and then inoculated i.p. with a lethal number of FBL3 leukemia cells. As shown in Fig. 6, all five mice immunized with LPS-activated spleen cells pulsed with Db126 peptide, none of five mice immunized with LPS-activated spleen cells alone, and one of eight mice inoculated with PBS were alive after tumor challenges. A statistical significance ($p < 0.01$) was found between the group immunized with the WT1 peptide and the group immunized with LPS-activated spleen cells alone, or the group inoculated with PBS alone. This experiment was repeated with similar results. In both the immune and nonimmune mice, ascites was observed 3 days after the i.p. inoculation of the tumor cells. In the nonimmune mice, the ascites continued to increase and the mice died. On the other hand, in the immune mice, the ascites gradually decreased afterward, and the mice completely rejected tumor challenges and survived. Spontaneous regression was occasionally observed in nonimmune mice. This regression is presumed to be due to spontaneous induction of CTLs specific for the Friend leukemia virus (FBL3 leukemic cells are transformed by this virus), since such CTL induction was not infrequently observed in C57BL/6 mice (42).

**No evidence of autoaggressive reactions in surviving mice that rejected tumor cell challenges**

WT1 expression in normal adult mice is limited to a few cell types in several tissues. Moreover, WT1 expression levels in these tissues are considerably lower than those in leukemia (13) and solid tumor (21) cells, suggesting a low risk of normal tissue damage as a result of immune responses to the WT1 protein. To evaluate the risk of autoaggression by immunization against self-WT1 peptide, the tissues of immunized mice were pathologically examined a few weeks after tumor cells had been eradicated. The lung and kidney of three mice were intensively examined because WT1 was mainly expressed in the mesothelial cells of the lung capsule and in the podocytes of the kidney glomeruli (Fig. 7). Both tissues showed normal structure and cellularity in all three mice examined, and no pathological changes caused by immune response, such as lymphocyte infiltration or tissue destruction and repair, were observed. These results showed that the CTLs against the Db126 peptide were ignorant of normal self-cells that express WT1 at physiological levels.

**Discussion**

The rationale for the efficacy of immunotherapy for cancer patients has been clinically demonstrated by the following findings (23): patients who develop significant GVHD (grade $\geq 2$) after allogeneic BMT have a significantly lower rate of relapse than patients with either no GVHD or grade I GVHD; patients who receive T cell-depleted marrow also have a higher rate of relapse after allogeneic BMT than patients who receive unmodified marrow; and donor lymphocyte infusion is effective for complete remission induction of relapsed leukemia patients after allogeneic BMT. This graft-vs-leukemia effect after allogeneic BMT provides the most convincing clinical evidence for the effectiveness of tumor immunity for cancer treatment.

The search for widely expressed tumor Ags as targets for MHC class I-restricted CTLs is of great importance for the development of new immunotherapeutic strategies.
of T cell-mediated immunotherapy for cancer patients. Reports on such tumor Ags have been increasing exponentially in recent years, and the results indicate that these Ags can be categorized into five groups (43, 44). Ags of the first category correspond to peptides derived from regions of ubiquitous proteins such as mutated ras (4) or p53 (5). Chimeric proteins that result from chromosomal translocation are also unique to tumor cells (45, 46). The second group of tumor Ags consists of tumor-specific shared Ags such as P1A in mice (6) and MAGE in humans (7, 8). The third group of tumor Ags includes differentiation Ags. A good example is tyrosinase, a melanocyte protein that gives rise to different peptides that are presented by either MHC class I or class II molecules (9). The fourth group of tumor Ags is made up of overexpressed tumor Ags. An Ag that is expressed in some normal tissues and overexpressed in tumors is HER-2/neu (10), which is found at high levels in about 30% of breast and ovarian cancers. The last group of tumor Ags includes Ags derived from oncogenic viruses. Thus, the WT1 protein is thought to be a tumor Ag corresponding to the fourth group of tumor Ags.

In the murine models of immunotherapy against WT1-expressing tumors described here, surviving mice that rejected tumor challenges by the immunization of the Db126 peptide did not demonstrate obvious organ damage. These results demonstrate that the CTLs against the WT1 protein can discriminate differences in WT1 expression levels between abnormally WT1-overexpressing tumor cells and physiologically WT1-expressing normal cells, resulting in the killing of tumor cells with no damage to normal tissues. As for the application of immunotherapy with WT1 protein to human cancers, the following evidence suggests that this immunotherapy is promising without damage to normal organs. We (12, 13) and others (14–17) have demonstrated that the wild-type WT1 gene is aberrantly overexpressed in almost all leukemia cells regardless of type of leukemia: whether it is acute myeloid leukemia, acute lymphoid leukemia, or chronic myeloid leukemia. The WT1 expression levels in leukemia cells are at least 10 times higher than those in normal CD34+ hematopoietic progenitor cells (13). This striking difference in WT1 expression levels between leukemia cells and normal hematopoietic progenitor cells is the basis for the reasoning that CTLs induced against the WT1 protein would not cause damage to normal hematopoietic progenitor cells. Furthermore, we have recently reported WT1 expression in 28 (82%) of 34 various types of solid tumor cell lines, including lung, gastric, colon, breast, and ovary cancer cell lines. High WT1 expression in fresh lung cancer tissues has also been reported (21). Our reports demonstrate that WT1 expression is significantly higher in cancer cell-rich tissues than in tissues appearing to be normal, confirming the abnormal overexpression of the WT1 gene not only in cultured tumor cells but also in fresh lung cancer cells. As mentioned earlier, the striking difference in WT1 expression levels between tissues appearing to be normal and cancer cell-rich tissues is also the basis for the reasoning that CTLs induced against WT1 protein would not cause damage to normal lung tissue.

The WT1 gene has been categorized as a tumor suppressor gene (3). However, we have proposed that the WT1 gene has basically two functional aspects, namely, that of a tumor suppressor gene and that of an oncogene, but that in leukemic cells it performs an oncogenic rather than a tumor suppressor gene function (18–20). The following findings support our proposal: 1) high expression of wild-type WT1 in almost all leukemic cells (12, 13), 2) an inverse correlation between WT1 expression levels and prognosis (12), 3) an increased WT1 expression at relapse compared with that at diagnosis in acute leukemia (47), 4) growth inhibition of leukemic cells by WT1 antisense oligomers (18), and 5) blocking of differentiation but induction of proliferation in response to G-CSF in myeloid progenitor cell line 32D c13 (19) and normal myeloid progenitor cells (20), both of which constitutively express WT1 by the transfection with the WT1 gene. Furthermore, it is suggested that the wild-type WT1 gene also has an oncogenic function in WT1-expressing solid tumors, since the WT1 gene is overexpressed in various types of solid tumor cells, including lung cancers, and since the suppression of WT1 gene expression by WT1 antisense oligomers inhibits the growth of solid tumor cells (21).

The loss of tumor-specific Ags followed by the escape from immune surveillance by CTLs is one of the major obstacles of the host’s immunological warfare against tumors. Since the WT1 protein plays an essential role in the growth of leukemic and solid tumor cells, loss of the expression of the WT1 protein, i.e., loss of the WT1 Ag, results in cessation of the proliferation of leukemic and solid tumor cells. Thus, immunotherapy directed against the WT1 protein would have little risk of escape from immune surveillance following loss of the WT1 Ag.

It has been well documented that tolerance to self-peptides is induced by deletion of self-reactive T cells in the thymus (48) as well as by deletion or exhaustion of such cells in the periphery (49), and that self-reactive T cells which have escaped deletion are functionally anergized or silenced by down-regulation of coreceptor molecules (50, 51). Since WT1 is a self-protein, it is considered to become tolerant in classical immunology. However, increasing evidence promoted us to accept that a large quantity of antigenic determinants of the self have not induced self-tolerance and thus that a substantial number of self-reactive clones must exist in healthy individuals and have the potential to elicit immune responses directed against tumors. These potentially self-reactive T cell clones are either anatomically secluded (52) or can be simply ignorant of their targets (53–55). It is probably possible to break tolerance especially if the self-proteins are not expressed at sufficient levels at the time and place of tolerance induction. The WT1 peptides are likely to be subdominant self-peptides so that the epitopes are probably ignored by the immune system under physiological conditions, although CTL precursors responsible for the WT1 peptides are present.

WT1 peptides that were predicted on the basis of the peptide library-based scoring system of MHC class I-binding peptides (25, 26, 29) actually showed comparatively higher binding affinity for H-2Db molecules, confirming that this scoring system is useful for finding candidates for MHC class I-binding peptides. Dyall et al. (56) designed a few artificial variants of MHC class I-binding self-peptides. Since these variant peptides are obviously foreign to the host immune system, a strong CTL response can be induced. Unlike weak T cell responses to self-MHC complexes, CTL responses to variant peptides can be sustained for a longer period without causing annihilation of the clones due to insufficient signals for cell division or survival (57, 58). Since a substantial fraction of such CTLs cross-reacts against nonmutated self-peptides expressed in tumor cells in much smaller amounts, immunization with variant peptides may be a more efficient method to induce CTLs against tumors. The scoring system for MHC class I-binding peptides should provide a convenient design of cross-reactive self-mimicking peptides for immunization.

We have recently reported that in vitro stimulation of HLA-A2.1-positive PBMC with WT1 peptides, Db126, RMFPNAPYL, or WH187, SLGEOQQYSV, both of which contain anchor motifs needed for binding to HLA-A2.1 molecules and actually bind to HLA-A2.1 molecules, elicits CTLs against each WT1 peptide (59). The CTLs specifically killed the WT1 peptide-pulsed target cells and endogenous WT1-expressing leukemic cells in an HLA-A2.1-restricted fashion. Thus, the WT1 peptide Db126 that was a shared
sequence between murine and human WT1 protein was immuno-
genic for the induction of CTLs in both mice and humans. These accumulated data obtained from both human and murine settings suggested a successful clinical application of WT1 protein-di-
rected immunotherapy for patients with leukemia and solid tumors. We are now planning clinical trials of this immunotherapy for patients with leukemia or lung cancer.

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