T-cell Immune Abnormality and Novel Immunotherapeutic Approach by Blocking the B7-H1–PD-1 Pathway Combined with WT1 Tumor Vaccine in Myelodysplastic Syndromes

Asaka Kondo

Department of Pathophysiological Management/Medical Oncology, Graduate School of Medicine, Nippon Medical School

Myelodysplastic syndromes (MDS) are hematologic malignancies caused by the neoplastic transformation of hematopoietic stem or myeloid progenitor cells and characterized by cytopenias, excessive apoptosis of hematopoietic cells, and a high risk of progression to acute myeloid leukemia. During disease progression, clonal blasts gain a more aggressive nature, whereas nonclonal immune cells become less efficient via an unknown mechanism[1]. Stem cell transplantation is the only curative treatment for MDS, but it is not suitable for elderly patients. B7-H1 (CD274) molecules, which are expressed on antigen-presenting cells and inhibit T-cell responses through programmed death-1 (PD-1) expressed on activated T cells, are expressed on various tumor cells, and B7-H1-expressing tumor cells evade attack by tumor-specific cytotoxic T lymphocytes (CTLs)[2]. We reported immune dysfunction associated with T cells in MDS, i.e., an increase in T-cell apoptosis, higher expression levels of PD-1 on circulating T cells, and higher levels of plasma-soluble interleukin-2 receptor[3,4]. Furthermore, we demonstrated that MDS blasts overexpress Wilms tumor gene WT1 mRNA[5], suggesting that the anti-WT1 immune response elicited by WT1 peptide vaccine may induce tumor regression in some patients. In the current study, we investigated B7-H1 expression on MDS blasts and analyzed the characteristics and inhibitory effects on T-cell immune responses of B7-H1$^+$ blasts. Finally, to develop new strategies, we are now analyzing a novel immunotherapy involving blockade of the B7-H1-PD-1 pathway in combination with WT1 tumor vaccine and/or inhibition of regulatory T cells.

First, we analyzed B7-H1 expression in 3 MDS cell lines, i.e., F-36P, OIH-1, and SKM-1, and on MDS blasts from 29 MDS patients, 32 patients with acute myeloid leukemia transformed from MDS (AL-MDS), and 10 hematologically normal individuals. A high level of B7-H1 expression at the mRNA and protein levels was detected only in F-36P cells using reverse transcription-PCR and flow cytometry (FCM), respectively. B7-H1 protein was not detectable on OIH-1 and SKM-1. Blasts from patients with high-risk MDS and AL-MDS expressed B7-H1 molecules more often compared with those from low-risk MDS patients. B7-H1 molecules were expressed in fewer than 5% of blasts in normal individuals. Furthermore, we found that the cytokines interferon (IFN)-γ and tumor necrosis factor (TNF)-α, which may be associated with MDS pathophysiology, induced B7-H1 expression on SKM-1 cells or blasts from MDS patients, and that B7-H1 induction by these cytokines was mediated by nuclear factor (NF)-κB activation, whose activation was observed in MDS patients, in particular in advanced disease.

Second, to investigate the characteristics of B7-H1$^+$ blasts, we analyzed proliferative advantage, i.e., cell cycle and colony formation, in B7-H1$^+$ and B7-H1$^-$ cell fractions in MDS blasts using FCM and the methylcellulose assay, respectively. B7-H1$^+$ MDS blasts had greater intrinsic proliferative capacity than B7-H1$^-$ MDS blasts when examined in both assays.

Third, to investigate the immunomodulatory effects of B7-H1$^+$ MDS blasts on T-cells, T-cell apoptosis and

proliferation were analyzed using FCM and the ^{3}H-thymidine incorporation assay, respectively, when T cells were cocultured with irradiated B7-H1^{+} MDS blasts, i.e., F-36P cells or B7-H1-expressing blasts from MDS patients, for 5 days. Blockade of the B7-H1–PD-1 pathway inhibited T-cell apoptosis and increased T-cell proliferation, indicating that B7-H1 molecules on MDS blasts inhibit T-cell responses.

Finally, we are now attempting to develop a new immunotherapeutic strategy in MDS using blockade of the B7-H1–PD-1 pathway. Blockade using anti-PD-1 antibody was reported to be associated with evidence of antitumor activity in some patients with refractory solid tumors[6]. However, the response rate to this immunotherapy was not satisfactory. Considering those results, we designed treatment regimens combining anti-B7-H1 or anti-PD-1 blocking antibody with WT1 peptide vaccine or combining inhibitory treatment of regulatory T cells with cyclophosphamide to enhance CTL attack. We are now investigating this new treatment in MDS blast-bearing mice.

B7-H1 is expressed on MDS blasts in advanced disease stage, and IFN-γ and TNF-α activate NF-κB, resulting in the induction of B7-H1 expression. B7-H1^{+} MDS blasts have an intrinsic proliferative advantage and evade tumor-specific CTLs, which may be involved in disease progression in MDS. Blockade of the B7-H1–PD-1 pathway may be highly synergistic in combination with WT1 tumor vaccine and/or inhibition of regulatory T cells. This combination immunotherapy may be become a new strategy to improve survival in elderly MDS patients.

References

1. Ishibashi M, Tamura H, Ogata K: Disease progression mechanism in myelodysplastic syndromes: insight into the role of the microenvironment. Leuk Res, in press.
2. Dong H, Strome SE, Salomao DR, et al.: Tumor-associated B7-H1 promotes T-cell apoptosis: A potential mechanism of immune evasion. Nat Med 2002; 8: 793–800.
3. Shioi Y, Tamura H, Yokose N, Sato C, Dan K, Ogata K: Increased apoptosis of circulating T cells in myelodysplastic syndromes. Leuk Res 2007; 31: 1641–1648.
4. Kondo A, Yamashita T, Tamura H, et al.: Interferon-γ and tumor necrosis factor-α induce an immunoinhibitory molecule, B7-H1, via nuclear factor B activation in blasts in myelodysplastic syndromes. Blood 2010; 116: 1124–1231.
5. Tamura H, Dan K, Yokose Y, et al.: Prognostic significance of WT1 mRNA and anti-WT1 antibody levels in peripheral blood in patients with myelodysplastic syndromes. Leuk Res 2010; 34: 986–990.
6. Brahmer JR, Drake CG, Wollner I, et al.: Phase I study of single-agent anti-programmed death-1 (MDX-1106) in refractory solid tumors: safety, clinical activity, pharmacodynamics, and immunologic correlates. J Clin Oncol 2010; 28: 3167–3175.