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A Novel Therapeutic Approach for Hematological Malignancies Based on Cellular Differentiation and Apoptosis

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Abstract

Hematological malignancies including acute leukemia, and multiple myeloma are disorders characterized by the accumulation of neoplastic hematopoietic cells, resulting in aggressive clinical manifestations with poor prognosis. The therapeutic approach to these disorders is basically chemotherapy for achieving complete remission based on the concept of total cell kill. However, severe side effects and complications such as serious infection and bleeding due to anti-cancer drugs are major problems in the clinical setting. In addition, repeated episodes of relapse of the disease may lead to refractory or chemotherapy-resistant disorders. These problems are occurred because anti-cancer agents have effects on both cancer cells and normal hematopoietic cells. The clinical evidences thus suggest the limitations of the chemotherapy for hematological malignancies: novel effective therapeutic approaches with less toxicity are therefore actively being sought. Differentiation-inducing therapy employing a physiologically active derivative of vitamin A, all-trans retinoic acid (ATRA), brought remarkably advances in the therapeutic outcome of APL at the end of last century. More recently, the clinical success of imatinib mesylate (STI571), potent competitive inhibitor of the Bcr/Abl protein tyrosine kinase, in the treatment of CML has focused enthusiasm toward molecular targeted therapy for the hematological malignancies. The therapeutic activity of these agents can be explained by their abilities to modify cellular growth, differentiation, and apoptosis in cells by activating unknown gene programs that molecular cellular proliferation. We have actively sought out new agents among natural products and cytokines with the ability to induce cellular differentiation and apoptosis. In this symposium, I will present our recent data of these novel compounds and their molecular mechanisms for inducing differentiation and apoptosis of hematological malignant cells.

1. Introduction

The therapeutic approach to hematological malignancies including leukemia and multiple myeloma is basically chemotherapy for achieving complete remission based on the concept of "total cell kill". However, severe side effects and complications such as serious infection and bleeding due to anti-cancer drugs are major problems in the clinical setting. In addition, repeated episodes of relapse of the disease may lead to refractory or chemotherapy-resistant disorders. The clinical evidences thus suggest the limitations of leukemia chemotherapy; novel effective therapeutic approaches with less toxicity are therefore actively being sought.

Differentiation-inducing therapy employing a physiologically active derivative of vitamin A, all-*trans* retinoic acid (ATRA), brought remarkable advances in the therapeutic outcomes of acute promyelocytic leukemia (APL) at the end of the last century [1]. In short, the therapeutic activity of ATRA in cases of APL can be explained by its ability to modify cellular growth, differentiation, and apoptosis in leukemic cells by activating unknown gene programs that modulate cellular proliferation. Recently, the clinical success of the Abl kinase inhibitor STI571 (imatinib mesylate) in the treatment of chronic myeloid leukemia (CML) has focused

renewed enthusiasm toward novel strategies for molecular targeted therapy [2]. However, recent clinical studies have revealed that most patients who received continuous treatment with ATRA and STI571 developed each agent-resistant disease [3-5]. Therefore, investigators have actively sought out new agents with the ability to stimulate cellular differentiation and induce apoptosis in the various types of cells associated with acute leukemia and multiple myeloma. Currently, we have found new agents among the natural products and cytokines with the ability to induce cellular differentiation and apoptosis. Furthermore, we established a human leukemia and myeloma model system in human GM-CSF-producing transgenic SCID and NOD/SCID mice transplanted with various malignant cells [6]. These *in vivo* models may become a useful preclinical tool for estimation of new agents to approve the clinical setting.

2. BMP Induces Apoptosis in Human Myeloma Cells

BMPs (bone morphogenetic proteins), members of the TGF-β superfamily, are a group of related proteins which are capable of inducing the formation of cartilage and bone, but are now regarded as multifunctional cytokines. However, little is known about their role in hematopoiesis. We have investigated that BMP-2 induces cell cycle arrest in the G1 phase and subsequent apoptosis in mouse B cell hybridoma HS-72 cells [7]. BMP-2 inhibited Rb phosphorylation by $p21^{CIP1/WAF1}$, resulting in the G1 cell cycle arrest. In addition, we identified a BMP-responsive sequence in the promoter region of $p21^{CIP1/WAF1}$ gene [8]. BMP-2 activated the transcription of by $p21^{CIP1/WAF1}$ inducing a binding of Smad4, Smad1, and activated BMP type I-receptor to the 29 base region in the promoter of $p21^{CIP1/WAF1}$ gene. Therefore, we next examined the effect of BMP-2 on human myeloma cells which are neoplastic disorder of terminally differentiated B-cells. BMP-2 induced apoptosis not only human myeloma cell lines but also in primary samples from patients with multiple myeloma *in vitro* [9]. BMP-2 caused cell cycle arrest in the G1 phase which was associated with accumulation of $p21^{CIP1/WAF1}$ and $p27^{KIP1}$, and the subsequent apoptosis of myeloma cells. Further analysis showed that BMP-2 induced down-regulation of $Bcl\text{-}X_L$ mediated the inactivation of STAT3. We conclude that BMP may have the potential to be one of the novel therapeutic agents for treatment in patients with multiple myeloma because of the beneficial effects on both myeloma cells and bone diseases [10].

3. Green Tea Component, Catechin, Rapidly Induces Apoptosis Of Myeloid Leukemic Cells Via Modulation of ROS Production

Green tea polyphenol, (-)-epigallocatechin-3-gallate (EGCG), has potent chemopreventive effects against various tumors, and green tea consumption might be effective for reducing the incidence of certain human cancers [11]. We investigated the effects of EGCG on the induction of apoptosis in leukemic cells *in vitro* and *in vivo*. EGCG suppressed the cellular growth of various myeloid leukemic cells via the induction of apoptosis. Interestingly, EGCG rapidly induced apoptotic cell death in retinoic acid (RA)-resistant APL, UF-1 cells [12] within 3 h, whereas other myeloid leukemic cells were less sensitive to EGCG. EGCG induced apoptosis in UF-1 cells in association with the loss of mitochondrial transmembrane potentials ($\Delta\Psi m$) and activation of caspase-3 and -9. Elevation of intracellular reactive oxygen species (ROS) production was also demonstrated during EGCG-induced apoptosis in UF-1 as well as fresh myeloid leukemic cells. An antioxidant, N-acetyl-L-cystein (NAC), significantly reduced ROS production and EGCG-induced apoptosis. In addition, preincubation with the reduced glutathione (GSH) synthesis inhibitor, buthionine sulfoximine (BSO), enhanced EGCG-induced apoptosis, suggesting that decreasing GSH levels accelerates the apoptotic effects of EGCG. Consistent with their sensitivity to EGCG, UF-1 cells had lower levels of GSH than did other leukemic cells. The results suggest that the modulation of GSH may be important for determining sensitivity to EGCG-induced apoptosis. In NOD/SCID mice transplanted with UF-1 cells, EGCG effectively inhibited tumor growth *in vivo*, and the number of mitosis among the cells significantly decreased in comparison to that of control mouse cells. These results suggest that EGCG has potential as a novel therapeutic agent for myeloid leukemia via induction of apoptosis mediated by modification of the redox system.

4. Homovanillic Acid Derivatives, Capsaicin, Induced Apoptosis of Leukemic Cells Mediated P53-Dependent Pathway

Capsaicin (*N*-vanillyl-8-methyl-1-nonenamide) is a homovanillic acid derivative and the principal pungent and irritating constituent of capsicum fruits. It has been suspected that spicy foods play some role in an erosion of gastric mucosa and human carcinogenesis. On the contrary, capsaicin has been reported that it causes a significant inhibitory effect on the adenoma-bearing mice. The tumor suppressor gene p53 is a gene-gatekeeper and mutated in the majority of human cancers, indicating that loss of p53 function may have an important role in tumorigenesis. We investigated the effect of capsaicin on the cellular proliferation, differentiation, and apoptosis of leukemic cells. Phosphorylation of Ser 15 of p53 and accumulation of p53 in the RA-sensitive APL, NB4 cells, were occurred in the treatment with capsaicin in a dose- and time-dependent manner. p53 acticvation by capsaicin induced the loss of $\Delta\Psi m$, induction of Bax protein, activation of caspase-3 and subsequent induction of apoptosis in NB4 cells. Marked contrast, capsaicin failed to inhibit cellular growth of leukemic cells with mutated p53. These results suggest that capsaicin induced apoptosis and cell cycle arrest in leukemic cells in association with activation of p53.

5. Conclusion

Patients with hematological malignancies often follow invariably fatal clinical courses. Recently, high-dose chemotherapy followed by hematopoietic stem cell transplantation frequently produces higher remission rates; however, it often causes serious clinical side effects and entails the risk of early mortality within a year, especially in elderly patients. Most patients ultimately relapse; therefore, novel therapeutic approach based on new insights into the pathogenesis of hematological malignancies and targeted important molecules for cellular proliferation are strongly desired. Therapeutic strategies of inducing cellular differentiation and apoptosis in APL cells by arsenic trioxide is one of the recent successful example for the clinical application of the natural compound [13,14]. Advantages of natural products and cytokines for application of clinical use are lack of toxicity. Therefore, these compounds and cytokines inducing apoptosis of malignant cells might be developed as a new potent anti-cancer agent for the management of hematological malignancies, particularly for the older and immunocompromised patients. In addition, these agents have potential to replace or augument the more cytotoxic agents currently used to treat the patients with hematological malignancies.

References

1. Tallman MS, Nabhan C, Feusner JH, Rowe JM. Acute promyelocytic leukemia: new mechanisms, strategies, and implications. *Blood.* 2001;99:759-767.
2. Goldman JM, Melo JV. Targeting the Bcr-Abl tyrosine-kinase in chronic myeloid leukemia. *N Engl J Med.* 2001;344: 1084-1086.
3. Kizaki M, Ueno Y, Yamazoe M, et al. Mechanisms of retinoid resistance in leukemic cells: Possible role of cytochrome P450 and P-glycoprotein. *Blood.* 1996;87:725-733.
4. Takayama N, Kizaki M, Hida T, Kinjo K, Ikeda Y. A novel mutation in the PML/RARa chimeric gene exhibits dramatically decreased ligand-binding activity and confers acquired resistance to retinoic acid in acute promyelocytic leukemia. *Exp Hematol.* 2001;29:864-872.
5. Gorre ME, Mohammed M, Ellwood K, et al. Clinical resistance to STI-571 cancer therapy caused by BCR-ABL gene mutation or amplication. *Science.* 2001;293:876-880.
6. Fukuchi Y, Kizaki M, Kinjo K, et al. Establishment of a retinoic acid resistant acute promyelocytic leukemia model in hGM-CSF transgenic SCID mice. *Br J Cancer.* 1998;78:878-884.
7. Yamato K, Hashimoto S, Okahashi N, et al. Dissociation of bone morphogenetic protein-mediated growth arrest and apoptosis of mouse B cells by HPV-16 E6/E7. *Exp Cell Res.* 2000;257:198-205.
8. Yamato K, Hashimoto S, Imamura T, et al. Activation of $p21^{CIP1/KIP1}$ promoter by bone morphogenetic protein-2 in mouse B lineage cells. *Oncogene.* 2001;20:4383-4392.
9. Kawamura C, Kizaki M, Yamato K, et al. Bone morphogenetic protein (BMP)-2 induces apoptosis in human myeloma cells with modulation of STAT3. *Blood.* 2000;96:2005-2011.
10. Kawamura C, Kizaki M, Ikeda Y. Bone morphogenetic protein (BMP)-2 induces apoptosis of human myeloma cells. *Leuk Lymphoma*. 2002;43:635-639.
11. Pisters AMW, Newman RA, Coldman B, et al. Phase I trial of oral green tea extract in adult patients with solid tumors. *J Clin Oncol.* 2001;19:1830-1838.
12. Kizaki M, Matsushita H, Takayama N, et al. Establishment and characterization of a novel acute promyelocytic leukemia cell line (UF-1) with retinoic acid resistant features. *Blood.* 1996;88:1824-1833.
13. Kinjo K, Kizaki M, Nuto A, et al. Arsenic trioxide (As2O3)-induced apoptosis and differentiation in retinoic acid-resistant acute promyelocytic leukemia model in hGM-CSF-producing transgenic SCID mice. *Leukemia.* 2000;14:431-438.
14. Muto A, Kizaki M, Kawamura C, et al. A novel differentiation-inducing therapy for acute promyelocytic leukemia with a combination of arsenic trioxide and GM-CSF. *Leukemia.* 2001; 15:1176-1184.