Chapter 17
Mylabris Phalerata (Chinese Blister Beetle) on Hematological Malignancies

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Abstract The therapeutic approach to hematological malignancies is based on chemotherapy using anticancer drugs, but such treatment has serious side effects, and some complications (e.g. serious infection and bleeding) associated with use of these drugs can be fatal. Therefore, investigators have sought out new less toxic agents targeted against the molecules responsible for the pathogenesis of the hematological malignancies. Natural compounds appear to be safer than some recently released anticancer drugs, and such compounds are promising for the development of novel compounds with improved clinical activities. Cantharidin (CTD) is the active ingredient of *Mylabris phalerata* (Chinese blister beetle), and is one of many natural products used in traditional Chinese medicine for cancer treatment. CTD is a selective inhibitor of PP1 (protein phosphatase 1) and PP2A, and as such is necessary for growth inhibition of tumor cells. In addition, the cytotoxic activity of CTD is likely to be associated with PP1 and PP2A activity. Therefore, a number of investigations of the effects of CTD on cancer cells have been carried out to date. Although CTD and its derivatives have been synthesized and examined in terms of their participation in antitumor processes in various cancer cell lines, there has been little progress in terms of clinical applications. Thus, it will be necessary to address the molecular modes of action of CTD in tumor cells, including hematological malignant cells. To address the molecular modes of action of CTD in tumor cells, several genes functionally related to cell proliferation or apoptosis were recently identified by cDNA microarray analysis in CTD-treated cells. It is possible that CTD will eventually contribute to the development of molecular-targeted therapies and individualized treatment strategies for the patients with hematological malignancies.

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17.1 Introduction

Natural product extracts have been proven to be a rich source of small molecules that display antitumor activity (Mann 2002; Gordaliza 2007). The search for improved cytotoxic agents continues to be important for the discovery of novel anticancer drugs. The structural diversity of natural compounds and their potential bioactivity have led to the isolation of several products isolated from plants, marine flora and microorganisms expected to provide "lead" compounds for the development of their therapeutic potential *via* molecular modification. The molecular modification of the functional groups of such lead compounds can generate structural analogs with anticancer effects. Modification processes have thus been able to advance the compounds towards their introduction into the clinical setting. For example, the main constituent of the plant *Catharanthus reseus* was the frontrunner among anticancer agents known as the vinca alkaloids (vincristine and vinblastine). Both of these drugs, which were introduced into clinical use in the late 1960s, and both have contributed to long-term remissions and cures of testicular teratoma, malignant lymphoma, lymphoblastic leukemia, and many other cancers (Lee 1999; Mukherjee et al. 2001). An example of a marine-derived anticancer agent developed for clinical use would be cytarabine (Adrian 2007), which is currently used to treat patients with lymphoma and leukemia, in particular acute myeloid leukemia (AML). Another agent, gemcitabine, was derived from a marine organism; gemcitabine has been shown to process significant therapeutic activity in patients with various types of solid tumors (Coseri 2009). In general, agents derived from natural compounds have contributed significantly to the successful treatment of several different types of cancers. Natural products or their structural relatives now account for approximately 50% of all drugs used as cancer chemotherapies. In addition, natural products from traditional Chinese medicine (TCM) can recently contribute to the development of molecular-targeted therapy for various malignancies (Chen and Liang 2010; Youns et al. 2010). Therefore, a very important area of research within the field of hematology/oncology is the routine examination of terrestrial plants and microorganisms aimed at the discovery of novel anticancer agents with more potent, more selective and less toxic compounds (Kizaki 2006).

17.2 Cantharidin (CTD)

CTD is a derivative of *Mylabris phalerata* (Chinese blister beetle) and a potent inhibitor of the protein phosphatase (PP) 2A (Li and Casida 1992; Honkanen 1993; Eldridge and Casida 1995; McCluskey et al. 2001; To 2004; Shan et al. 2006; Rauh et al. 2007). The basic structure of CTD consists of a 7-oxobicycloheptane, a dicarboxylic acid, and an anhydride (Fig. 17.1) (Honkanen 1993; Shan et al. 2006; Rau et al. 2007). The use of dried Chinese blister beetles as an anticancer agent extends back through more than 2,000 years of TCM (Wang 1989; Rauh et al. 2007). To

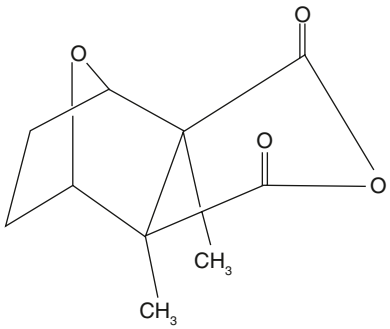

Fig. 17.1 Chemical structure of cantharidin

date, CTD has been reported in the literature to exert an effect on the control of cell-cycle regulation and on the cellular growth of tumor cells with transformed SV40 antigen (Clarke et al. 1993; Sontag et al. 1993; Janssens and Goris 2001). Recent studies have shown that CTD induces apoptosis in the following types of cancer; human hepatoma, pancreatic cancer, colon cancer, oral buccal carcinoma, and human leukemia cells (Peng et al. 2002; Chen et al. 2002, 2008; Williams et al. 2003; Huh et al. 2004; Huan et al. 2006; Kok et al. 2006a, b; Li et al. 2010). In clinical trials, CTD was found to be toxic to mucous membrane tissues, including those in the gastrointestinal tract, urethra, and kidney with a dose-limiting factor of renal toxicity (Wang 1989). In addition, it has reported that there are cases of CTD poisoning following ingestion of the beetles (Tagwireyi et al. 2000). The symptoms of CTD poisoning are directed towards the gastrointestinal tract and the genitourinary tract. In this case report, the patient presented with lower abdominal pain, hematuria, proteinuria and oligouria (Tagwireyi et al. 2000). Norcantharidin was synthesized to reduce renal toxicity, and is currently being clinically evaluated for use in colon cancer patients (Wang 1989). Furthermore, CTD and its analogues have exhibited therapeutic effects against primary hepatoma and esophageal carcinoma without being associated with the suppression of bone marrow (Wang 1989; Li et al. 2010). Recent large systemic review and meta-analysis in China provide the important information that TCM including CTD can be possible therapeutic option for the patients with hepatoma (Wu et al. 2009). However, this should be necessary to confirm by well-conducted prospective randomized clinical trial.

17.3 Effects of CTD on Tumor Cells

17.3.1 Protein Phosphatase Activity

The molecular mechanisms of CTD responsible for its underlying anticancer effects remain unknown. However, CTD is known to be a PP1 (protein phosphatase 1) inhibitor as well as a PP2A (protein phosphatase 2A) inhibitor (Li and Casida 1992;

Honkanen 1993; Eldridge and Casida 1995; McCluskey et al. 2001; To et al. 2004; Shan et al. 2006; Rauh et al. 2007). Such activity appears necessary for CTD to induce growth inhibition. PP2A is a ubiquitous enzyme involved in the dephosphorylation of the serine and threonine residues of cellular phosphoproteins, and thereby is also involved in the moderation of cellular proliferation (Janssens and Goris 2001). Protein phosphatases are also involved in the regulation of multiple cellular processes including apoptosis, cell cycle regulation, and various signal transduction pathways. PP2A has been thought to be a tumor suppressor, because inhibition of PP2A can induce the phosphorylation and activation of various substrate kinases (Millward et al. 1999; Janssens et al. 2005). Members of the MAPK family (i.e. ERK, JNK, and p38 MAP kinase) are direct substrates of PP2A (Millward et al. 1999). In general, the activation of MAPKs promotes the growth of tumor cells; however, continuous activation of these kinases can also inhibit proliferation and induce apoptosis in many tumor cells (Zhang and Liu 2002). PP2A inhibits the signal transduction pathway of ERK, thus affecting the calcium/calmodulin- and ceramide-dependent pathways of cellular growth in tumor cells (Li et al. 2010). It has also been reported that MAPK-family kinases such as JNK and ERK become active after CTD stimulation, which results in an increase in the caspase-3-mediated apoptosis of tumor cells (Huh et al. 2004; Schweyer et al. 2007). PP4 is a family of serine/threonine phosphatases that regulate a variety of cellular functions not regulated by PP2A. It has been reported that NF-κB signaling as well as the mammalian target of rapamycin (mTOR) pathways are regulated by PP4, and CTD possesses similar inhibitory activity to that of PP4 (Cohen et al. 2005). In addition, it has been reported that CTD inhibits JNK, but neither ERK nor p38 in pancreatic cancer cells, suggesting that CTD exerts its anticancer effects *via* a JNK-dependent pathway (Li et al. 2010). The findings to date suggest that CTD activates the MAP-kinase pathways, which results in a significant increase in caspase-mediated apoptosis in tumor cells. In addition, it is well known that okadaic acid, another PP2A inhibitor, induces the apoptosis of various types of tumor cell including leukemia, myeloma, hepatoma, and intestinal epithelial cells (Ishida et al. 1992; Kang et al. 1996; Lamboley et al. 2000; Ray et al. 2005).

17.3.2 Microarray Analysis

To identify candidate genes that affect the sensitivity of tumor cells to CTD, several microarray analyses were performed. Efferth (2005) and Rauh et al. (2007) identified 21 of 9,706 genes, the mRNA expression of which in 60 tumor cell lines correlated with the sensitivity of tumor cells to CTD. The majority of these genes are involved in DNA damage response, DNA repair, and apoptosis (Efferth 2005). From these CTD-related 21 genes identified by Efferth, important genes related to DNA repair and induction of cellular apoptosis is listed (Table 17.1). Among the panel of CTD-regulated genes, *PPP1R13B* is an interesting gene, because CTD is an inhibitor of PP1 and PP2A. PPP1R13B is the regulatory subunit 13B of PP1

Table 17.1 Important genes induced by cantharidin. (from Efferth 2005 with modification)

Symbols	Gene name	Function
SUSP1	SUMO-1-specific protease	Maturation and activation of Sumo-1. Related to DNA repair and induction of apoptosis
RPA2	Replication protein A2	DNA damage recognition, DNA replication, recombination and repair
CASP4	Caspase 4, apoptosis-related cysteine peptidase	Apoptosis execution. Stress-inducing agents activate endoplasmic reticulum-localized caspase 4
PPP1R13B	Protein phosphatase 1, regulatory (inhibitor) subunit 13B	Interaction with protein phosphatase 1. DNA repair and regulation of apoptosis
PDLIM1	PDZ and LIM domain 1	Cytoskeletal adaptor for proteins *via* its LIM and PDZ domains. Regulation of apoptosis *via* interaction with Fas-, p53-, NF-κB-, and Myc-signaling
UNR	N-RAS-related gene	RNA-binding protein; coordinated up-regulation with *N-RAS*. Regulation of apoptosis
HSBP1	Heat shock binding protein 1	Negative regulator of stress responses by binding to HSP70. DNA repair and regulation of apoptosis

Out of 21 genes identified by Efferth, important genes related to DNA repair and induction of cellular apoptosis are listed. These genes were identified by COMPARE and false discovery rate analyses whose mRNA expression in the panel of 60 human tumor cell lines of the Developmental Therapeutics program of the National Cancer Institute (USA) correlated with IC_{50} values for cantharidin (Efferth 2005)

and plays a central role in the regulation of induction of cellular apoptosis *via* interaction with p53, suggesting that PPP1R13B has related to CTD-induced DNA repair and apoptosis. These results suggest that oxidative stress response genes reduce the activity of CTD by inducing DNA strand breaks, which may induce the apoptosis of tumor cells in a p53- and Bcl-2-dependent manner. It has also been reported that CTD induced the expression of Bax protein, but down-regulated the expression of Bcl-2 and survivin in A549 cells resulting in the induction of apoptosis (Liu and Chen 2009). Another group used HL-60 myeloid leukemia cells treated with CTD to demonstrate the up- and down-regulation of mRNA expression levels of 2,087 genes by cDNA microarrays (Zhang et al. 2004). That group reported that the CTD-treated cells did not exhibit any decreases in the expression of genes coding for proteins involved in DNA repair, DNA replication, or proteins with oncogenic activity. Furthermore, these cells overexpressed genes that encode growth inhibitory proteins such as *BTG2* and *MCP-3*, and proapoptotic genes. They also observed a down-regulation in the expression of multidrug resistance-associated protein genes in CTD-treated HL-60 cells, suggesting that CTD may be related to the increased expression of genes that modulate drug sensitivity in tumor cells.

17.3.3 DNA Damage and Repair Induced by CTD

The microarray analyses identified several apoptosis- and cell cycle regulated-genes involved in CTD-induced growth inhibition of tumor cells. In addition, DNA damage and repair correlated with the IC_{50} values for CTD in many tumor cell lines (Pang et al. 2007). Recently, Efferth et al. reported that CTD induces apoptosis in various leukemic cells *via* a p53-dependent mechanism (Efferth et al. 2005). The phosphorylation of p53 stabilizes the protein; therefore, PP1 and PP2A inhibitors such as CTD may synergistically enhance p53 activity. CTD inhibits the expression of anti-apoptotic Bcl-2 protein in leukemic cells, suggesting that a DNA damage-triggered mitochondrial pathway is also involved. Moreover, CTD causes both DNA single- and double-strand breaks in a time-dependent manner (Rauh et al. 2007). The nuclear DNA PolB (polymerase β) is a key enzyme in base excision repair (Efferth et al. 2005). This enzyme was found to be correlated with increased cell survival in CTD-treated cells, suggesting that the increased DNA strand breakage and DNA repair were related to a decrease in cellular sensitivity to CTD. Interestingly, it has reported that the synthetic integration of CTD-derived demethylcantharidin with classical platinum-based cytotoxic drug shows superior anticancer effect (Pang et al. 2007). This novel compound caused additional DNA damage in cancer cells suggesting that drug-herb interaction can provide novel efficient anticancer agent.

It has been also reported that the CTD-induced growth inhibition of tumor cells was dependent on the induction of oxidative stress, resulting in the induction of apoptosis and cell cycle arrest (Rauh et al. 2007). This oxidative stress initiated by CTD may cause direct DNA damage and p53-dependent apoptosis in tumor cells. However, Li et al. (2010) reported that CTD increased reactive oxygen species (ROS) levels in pancreatic cancer cells, and growth inhibition remained unaffected by the treatment with CTD. These results suggest that CTD may induce apoptosis and cell cycle arrest in pancreatic cancer cells in an oxidative stress-independent manner.

17.4 Effects of CTD on Malignant Hematological Cells

17.4.1 Therapeutic Approaches to the Treatment of Leukemia

Significant advances in molecular biology and therapies for hematological malignancies have been made over the last decade. Therapeutic approaches to the treatment of hematological malignancies such as leukemia, malignant lymphoma, and multiple myeloma are basically chemotherapies to eradicate malignant cells. In the past two decades, clinical research aimed at improving the cure rate for patients with hematological malignancies has focused primarily on increasing cytotoxic drug delivery with the aim of maximizing the number of tumor cells eradicated based on a concept of referred to as “total cell kill” (Skipper 1974). However, se-

vere side effects and complications due to anticancer drugs remain major problems in clinical practice. In addition, relapses are usually refractory to chemotherapy and are associated with a poor prognosis. Therefore, chemotherapy for hematological malignancies is limited by the development of drug resistance in tumor cells, adverse side effects, and myelosuppression. These serious clinical issues point to some of the limitations of current therapeutic strategies for the treatment of hematological malignancies. Therefore, novel more effective therapeutic approaches with less toxicity are still needed. Recent advances in the clarification of the molecular pathogenesis of leukemia have led to development of novel therapeutic approaches for the treatment of both AML and chronic myeloid leukemia (CML).

AML is a heterogeneous group of malignant disorders of hematopoietic progenitor cells marked by an accumulation of granulocyte and monocyte precursors in the bone marrow and peripheral blood. Despite scientific advances in our understanding of the epidemiologic, genetic, and biological features of AML, the disease remains fatal in a majority of patients, especially older individuals. In the 1980s, the use of all-*trans* retinoic acid for differentiation-inducing therapy in patients with acute promyelocytic leukemia was proposed by Professor Wang in Shanghai (China); subsequent therapeutic strategies for the treatment of leukemia yielded dramatic improvements in clinical outcome (Huang et al. 1988). Therapeutic strategies for inducing cellular differentiation and apoptosis in acute promyelocytic leukemia cells using all-*trans* retinoic acid and arsenic trioxide first described in China are one recent successful examples of the clinical application of natural compounds (Tallman et al. 2002). Recently, more specifically targeted agents have been developed for the treatment of AML, including anti-CD33 antibodies and immunoconjugate drugs, inhibitors of multidrug resistance proteins, farnesyl transferase inhibitors, tyrosine kinase inhibitors, histone deacetylase, and proteasome inhibitors (Grant 2009). However, these targeted-therapy candidates have yet to be translated into clinical application. In addition to conducting therapeutic trials, it will be important to identify other novel and highly specific therapeutic agents in parallel with our evolving understanding of the biology of AML.

CML is a clonal disease characterized by the presence of the Philadelphia chromosome and its oncogenic product, BCR-ABL, a constitutively active tyrosine kinase. Over the past three decades, several effective strategies for the treatment of CML have been developed (Valent 2010). IFN-α (interferon-alpha) is a cytoreductive agent that has been widely used to control CML. However, most patients treated with IFN-α do not exhibit a long-lasting response (Goldman 2009); the standard curative approach to CML is hematopoietic stem cell transplantation (HSCT) (Venepalli et al. 2010). However, HSCT can only be offered to a small subset of patients. Notably, HSCT is associated with enhanced disease-free survival, but also with transplant-related mortality and occasionally high morbidity (Venepalli et al. 2010). The approach to treating CML was revolutionized by the introduction of imatinib mesylate, a BCR-ABL tyrosine kinase inhibitor as a molecular-targeted therapy (Goldman 2001). The clinical use of specific BCR-ABL inhibitors has resulted in a significantly improved prognosis, response rate, overall survival, and patient outcome in CML patients compared to that achieved with previous therapeutic regimens. However, the complete

eradication of CML in patients receiving imatinib mesylate was limited by the emergence of resistance, mostly due to mutations in the ABL kinase domain, and to a lesser extent by molecular residual disease after treatment. The second-generation BCR-ABL tyrosine kinase inhibitors, nilotinib and dasatinib, have shown significant activity in clinical trials in patients intolerant or resistant to imatinib therapy, except in those patients with the T315I *BCR-ABL* mutation (Agrawal et al. 2010).

17.4.2 Effects of CTD on Leukemia

Epidemiological investigation and laboratory studies have indicated that bioactive natural compounds play an important role in the treatment of many cancers. Therefore, investigators have actively sought out new agents that will potentially yield positive clinical outcomes while inducing less toxicity than previous agents; these candidate agents are derived from a variety of chemical compounds, and from various natural products. Along these lines, we have carried out and reported studies of various bioactive agents derived from natural compounds that induce apoptosis, a basic molecular mechanism that takes place in human leukemia and myeloma cells (Ito et al. 2005; Nakazato et al. 2005a, b; Shimizu et al. 2006; Xian et al. 2007; Sagawa et al. 2008; Nakaya et al. 2010).

In the clinical setting, CTD has been used for the treatment of hepatocellular carcinoma and leukemia. However, renal toxicity and suppressive effects on bone marrow limit its use. To reduce toxicity, several modified CTD analogues with antitumor effects have been chemically synthesized (Lin et al. 2000). Recently, Kok et al. (2006b) synthesized a number of CTD analogues and screened them for possible cytotoxic effects using a panel of cancer cell lines. The focus of that study was on the electron distribution of molecules and the core structure of CTD with diimide and the 6-position trifluoromethroxy group. A new CTD analogue designated as CAN 032 was synthesized, and then CAN 037 was subsequently generated by modifying the methyl group at the 6-position (Kok et al. 2006b). This new compound strongly induced apoptosis in the myeloid leukemia cell line KG-1 *via* the induction of caspase-3, -8, and -9 activity. Furthermore, CTD induced apoptosis in the human lymphoid leukemia cell line CCRF-CEM in a p53-dependent manner. That study by Kok and colleagues demonstrated that CTD causes oxidative stress provoking DNA damage and p53-dependent cell death (Kok et al. 2006b).

Recent stem cell biology studies have classified leukemic stem cells (LSCs) as associated with a number of different types of leukemia; LSCs are a major focus of current research interest (Lane and Gilliland 2010). LSCs are an important target for the treatment of leukemia, and failure to eradicate these very primitive cancer cells is a common cause of relapse in leukemia patients. Therefore, improved understanding of the biology of LSCs, and of the differences between normal hematopoietic and leukemic stem cells is likely to lead to the development of novel therapies as well as to increase in patient survival. Recently, it has been shown that CTD targets primary AML stem and progenitor cells in contrast to conventional

chemo-therapeutic agents such as cytarabine and daunorubicin, which primarily target differentiated and cycling leukemic cells (Dorn et al. 2009). Dorn et al. reported that CTD reduced levels of the LSC target gene *HLF* at the protein level, and CTD also reduced the incidence of other leukemia-associated gene mutations such as activating mutations of *FLT-3* (Dorn et al. 2009). Constitutive active FLT-3 activates STAT5, causing an expansion of the hematopoietic stem cell pool and inhibiting myeloid differentiation *via* C/EBPα down-regulation (Schuringa et al. 2004; Chung et al. 2005; Wierenga et al. 2006). It was found that CTD inhibited IL-3-induced STAT5 phosphorylation in human AML stem and progenitor cells, suggesting that STAT5 is the direct target of CTD. From these experimental results, it appears likely that CTD could have a beneficial therapeutic effect on LSC-associated pathways, leading to potent eradication of LSCs and thereby a reduction in tumor burden.

17.4.3 Therapeutic Approaches to Multiple Myeloma

Multiple myeloma is characterized by the latent accumulation of secretory plasma cells with a low proliferative index and an extended life span in the bone marrow. Conventional therapy for multiple myeloma involves combinations of vincristine,melphalan, cyclophosphamide,doxorubicin (Adriamycin), and prednisone or dexamethasone (Palumbo and Rajkumar 2010). Patients younger than 65 years are usually given high-dose melphalan with autologous stem cell support, and older patients or those who cannot tolerate such intensive treatment are given standard-dose oral melphalan and dexamethasone. However, these treatments are associated with low remission rates, short survival times, and the development of drug resistance (Kumar et al. 2008). Chemo-resistance remains a major therapeutic challenge in the treatment of multiple myeloma.The precise mechanism underlying chemo-resistance in multiple myeloma is not clear, but one of the main contributors to both chemo-resistance and pathogenesis is thought to be the activation of NF-κB and STAT3 and the dysregulation of apoptosis (Hideshima and Anderson 2002). Recently, novel agents such as bortezomib, thalidomide, and lenalidomide, which target myeloma cells and their microenvironments, have shown remarkable activity against refractory and chemo-resistant cases in early clinical trials, and prolonged progression-free and overall survival of multiple myeloma patients (Weber 2003; Yasui et al. 2006). Progression and chemo-resistance are thought to involve interleukin (IL)-6, the expression of which is induced by NF-κB, *via* its regulation of the growth and survival of myeloma cells (Hideshima and Anderson 2002). IL-6 leads to the constitutive activation of STAT3, which in turn results in the expression of high levels of anti-apoptotic Bcl-xL and Mcl-1 proteins (Catlett-Falcone et al. 1999; Zhang et al. 2002). Thus, both the constitutive activation of NF-κB and STAT3 play an important role in chemo-resistance, and it is expected that the inhibition of NF-κB and STAT3 may overcome such chemo-resistance.

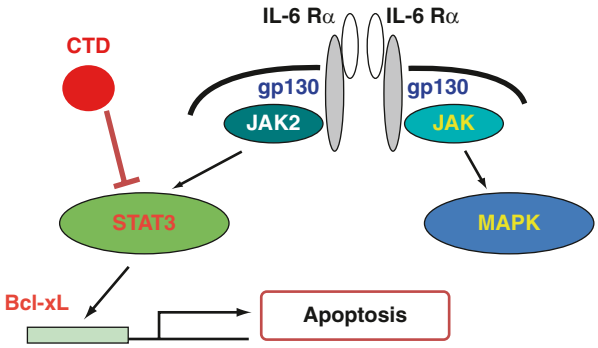

Fig. 17.2 Mechanisms of cantharidin (CTD)-induced apoptosis in human myeloma cells. CTD inhibited the phosphorylation of STAT3 (signal transduction and activator of transcription 3) and down-regulated the expression of anti-apoptotic Bcl-xL protein. STAT3 directly bound and activated the transcription of the *Bcl-xL* gene promoter. Thus, CTD induced apoptosis of human myeloma cells *via* the down-regulation of Bcl-xL and modulation of the STAT3 signaling pathway

17.4.4 Effects of CTD on Myeloma Cells

The use of natural agents may increasingly allow us to overcome treatment resistance without incurring some of the debilitating side effects of conventional chemotherapy. We have reported that CTD inhibited the cellular growth of human myeloma cell lines as well as that of myeloma cells freshly isolated from patients (Sagawa et al. 2008). Cultivation with CTD induced the apoptosis of myeloma cells in a cell-cycle-independent manner. Treatment with CTD induced caspase-3, -8, and -9 activity, which was in turn completely blocked by the respective caspase inhibitors. CTD inhibited the phosphorylation of STAT3 at the tyrosine 705 residue and down-regulated the expression of the anti-apoptotic Bcl-xL protein. STAT3 directly bound and activated the transcription of the *Bcl-xL* gene promoter, resulting in the induction of the expression of Bcl-xL in myeloma cells (Fig. 17.2) (Sagawa et al. 2008). Thus, it is clear that CTD-induced apoptosis in human myeloma cells may be mediated by the induction of anti-apoptotic proteins, and that CTD may has the potential to become a new therapeutic agent in the field of signal transduction therapies.

17.5 Conclusion

Patients with hematological malignancies such as leukemia and multiple myeloma often face a fatal clinical outcome. High-dose chemotherapy followed by hematopoietic stem cell transplantation has produced increasingly high remission rates, but this approach often causes serious clinical side effects and incurs the risk of early mortality, especially in elderly patients. Most patients ultimately relapse; therefore, new thera-

peutic approaches based on novel insights into the pathogenesis of hematological malignancies and that target molecules involved in cellular growth are needed. Natural products may play an important role in the development of novel drugs, in particular those for the treatment of cancer. The advantage of natural products for clinical application is the potential lack of toxicity. Therefore, it is hoped that compounds that induce apoptosis in cancer cells might be developed as new potent anticancer agents for the management of hematological malignancies, particularly in older patients. In order to yield successful clinical applications, target molecules from natural products will need to be identified for the development of rational treatment strategies.

Recently, many studied of the potential anticancer effects of CTD and its derivatives have been reported. CTD is recognized as a potent and selective inhibitor of PP1 and PP2A in tumor cells. The effects of CTD and its derivatives on cancer cell signal transduction pathways have investigated and the molecular mechanisms of CTD have gradually been revealed; however, the precise mechanisms of the above mentioned anticancer activities of CTD remain unclear. The molecular mechanisms of CTD and its derivatives still need to be investigated *in vitro* and *in vivo*; future studies will help elucidate the details of CTD-induced anticancer effects. Additional chemical modifications and the development of new CTD analogues are also needed, since there is great potential for molecules to be developed with reduced toxicity that retain antitumor- and target-related efficacy.

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