Arsenic trioxide, a therapeutic agent for APL

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Acute promyelocytic leukemia (APL) is an interesting model in cancer research, because it can respond to the differentiation/apoptosis induction therapy using all-trans retinoic acid (ATRA) and arsenic trioxide (As_2O_3). Over the past 5 years, it has been well demonstrated that As_2O_3 induces a high complete remission (CR) rate in both primary and relapsed APL patients (around 85~90%). The side effects are mild to moderate in relapsed patients, while severe hepatic lesions have been found in some primary cases. After CR obtained in relapsed patients, chemotherapy in combination with As_2O_3 as post-remission therapy has given better survival than those treated with As_2O_3 alone. The effect of As_2O_3 has been shown to be related to the expression of APL-specific PML–RARα oncoprotein, and there is a synergistic effect between As_2O_3 and ATRA in an APL mouse model. Cell biology studies have revealed that As_2O_3 exerts dose-dependent dual effects on APL cells. Apoptosis is evident when cells are treated with 0.5~2.0 μM of As_2O_3 while partial differentiation is observed using low concentrations (0.1~0.5 μM) of the drug. The apoptosis-inducing effect is associated with the collapse of mitochondrial transmembrane potentials in a thiol-dependent manner, whereas the mechanisms underlying APL cell differentiation induced by low dose arsenic remain to be explored. Interestingly, As_2O_3 over a wide range of concentration (0.1~2.0 μM) induces degradation of a key leukemogenic protein, PML–RARα, as well as the wild-type PML, thus setting up a good example of targeting therapy for human cancers. *Oncogene* (2001) **20,** 7146–7153.

Keywords: arsenic trioxide (AS_2O_3); acute promyelocytic leukemia (APL); apoptosis; differentiation; PML-RARα

Introduction

Arsenic has long been known to act as a carcinogen, involved in human skin, lung, liver, kidney and urinary bladder cancers (Huff *et al.*, 2000). Paradoxically, arsenic has also long been demonstrated to have anti-cancer activity in some cases. In traditional Chinese medicine, arsenic trioxide (As_2O_3) was recorded in the Compendium of Materia Medica by Mr Li Shi-Zhen (1518–1593). In Western medicine, arsenous oxide (Fowler's solution) was used as a treatment of choice for chronic myeloid leukemia (CML) in the 19th century. As Osler stated in his 1892 textbook of medicine, 'There are certain remedies that have an influence upon the disease. Of these, arsenic, given in large doses, is the best. I have repeatedly seen improvement under its use.' However, due to toxic side effects of long-term heroic-dose of oral arsenic in most patients, and with the advent of modern radio-therapy and chemotherapy, the arsenic treatment for CML was given up in Western medicine (Kwong and Todd 1997; Tamm *et al.*, 1998).

Recently, a discovery on the therapeutic effect of As_2O_3 in acute promyelocytic leukemia (APL) has revived this ancient drug (Chen *et al.*, 1996; Mervis, 1996; Look, 1998; Gallagher, 1998; Conrad, 1999; Warrell, 1999; Slack and Rusiniak, 2000; Wang and Chen, 2000; Chen *et al.*, 2001). APL is a special subtype of acute myeloid leukemia (AML), characterized by the specific chromosomal translocation t(15;17) with resultant leukemogenic PML–RARα fusion gene, and the clinical response to differentiation therapy using *all-trans* retinoic acid (ATRA) (Melnick and Licht, 2000). Over the past several years, As_2O_3 as an effective salvage treatment for relapsed and/or refractory APL patients has been confirmed worldwide. Recently, this drug was formally approved by the State Drug Administration (SDA) in China (1999) and the Food and Drug Administration (FDA) of the United States of America (Cohen *et al.*, 2001). Encouraged by such a bedside discovery, mechanisms of action of As_2O_3 have been actively addressed by many groups. The potentials of As_2O_3 in the treatment of cancers other than APL are also under exploration. Here, we shall discuss the recent advances in the clinical practices and the mechanisms of action of As_2O_3 in APL.

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Clinical practice of As_2O_3 in the treatment of APL

Clinical efficacy and pharmacokinetics

The recent clinical trial of As_2O_3 was started in 1971. A group from the First Hospital affiliated with the Harbin Medical University, China, used this compound through intravenous administration in more than 1000 cases of different types of cancer. Therapeutic effects were observed in several cancer types, including CML, lymphoma, esophageal cancer, and particularly APL. The preliminary result in APL was reported in 1992 (Sun et al., 1992). A preparation containing arsenic with a trace amount of mercury chloride was administered at a dose of 10 mg As_2O_3/day and could induce clinical complete remission (CR) in 21 out of 32 (65.6%) patients without major toxic side effects. In 1996, two reports were made from China using purified As_2O_3 (0.16 mg/kg/day). The Harbin group (Zhang et al., 1996) showed that As_2O_3 treatment achieved CR in 52.4% (22/42) relapsed APL patients. The Shanghai Institute of Hematology (SIH) reported an even better result among 15 patients relapsed after ATRA and chemotherapy, with CR achieved in nine of 10 patients treated with As_2O_3 alone and five of five patients treated with As_2O_3/chemotherapy combination. The treatment duration for induction of remission was 28–54 days. Of note, the only non-responder in the SIH series had leukemic cells losing t(15;17) and PML–RARα gene expression at relapse, although the disease was a typical APL at initial diagnosis, and the first CR was induced by ATRA (Chen et al., 1996; Shen et al., 1997).

The pharmacokinetic studies were performed among eight relapsed patients by measuring plasma drug levels with gas chromatography. The results showed that after a peak level of 6.85 (5.54–7.30) μmol/L, plasma arsenic was rapidly eliminated, with a $t_{1/2\alpha}$ of 0.89 (SD: 0.29) h and a $t_{1/2\beta}$ of 12.13 (SD: 3.31) h. Hence, over most times during As_2O_3 treatment, plasma arsenic levels fluctuated between 0.1–0.5 μM. Such a pharmacokinetic behavior did not alter after continuous use of As_2O_3. Increased amounts of arsenic appeared in the urine with a daily excretion accounting for approximately 1–8% of the total daily dose used. Arsenic contents in hair and nails were increased during treatment and declined after withdrawal of the drug (Chen et al., 1996; Shen et al., 1997).

The Memorial Sloan-Kettering Cancer Center of New York was the first to use As_2O_3 in the treatment of APL patients in Western countries. In the first report (Soignet et al., 1998), eleven of the 12 patients had a CR after treatment with 0.06–0.2 mg As_2O_3/kg/day for 12–39 days. A more recent U.S. multicenter study performed in 40 patients achieved a CR rate of 85% (Cohen et al., 2001). In addition, the effectiveness of As_2O_3 in the treatment of APL was also reported by some small-scale studies (Huang et al., 1998; Agis et al., 1999; Galimberti et al., 1999; Ohnishi et al., 2000; Zhang et al., 2000; Lin et al., 2000a).

Recently, a trial using low dose As_2O_3 (0.08 mg/kg/day) was carried out in SIH (Shen et al., 2001). Among 20 cases studied, a similar CR rate (80%) was achieved as compared to that attained with a conventional dose. Pharmacokinetic study was conducted in six patients given low dose As_2O_3. The mean C_{pmax} was 2.63 (range, 1.54–3.42) μmol/L, nearly half of the levels with a standard dose, and the ranges of $t_{1/2\alpha}$ and $t_{1/2\beta}$ were 1.2–2.7 h and 6.23–14.9 h, respectively. Of note, the arsenic concentrations in the plasma of bone marrow (BM) in five patients 24 h after administration of the drug at low dose were 0.061–0.49 μmol/L.

Improvement of bleeding diathesis with As_2O_3 treatment

Clinically, in patients with APL, the disease is frequently accompanied by a bleeding syndrome, which is often exacerbated by chemotherapy and causes death at the early stage of treatment. It was suggested that the aberrant expression of tissue factor (TF) in APL cells is at least one of the mechanisms underlying the pathogenesis of the APL coagulopathy (Barbui et al., 1998; Cheng et al., 1999; Tallman, 1999). In APL patients receiving As_2O_3 treatment, the improvement of disseminated intravascular coagulation (DIC) or hyperfibrinolysis paralleled the amelioration of bleeding symptoms, the correction of plasma fibrinogen level, and the decrease in membrane procoagulant activity (PCA) as well as TF contents of APL blasts (Zhu et al., 1999; Agis et al., 1999). Consistent with the in vivo findings, the membrane PCA and TF at both protein and mRNA levels in APL cell line NB4 were rapidly down-regulated by 1 μM As_2O_3 (Zhu et al., 1999; Ohsawa et al., 2000).

Toxic side effects

During As_2O_3 treatment in APL patients, there exists no BM depression. Instead, As_2O_3 induces leukocytosis in about 50% of patients (Shen et al., 1997; Soignet et al., 1998, Niu et al., 1999; Camacho et al., 2000). These patients can even develop retinoic acid syndrome (RAS)-like symptoms such as fever, skin rash, edema, which were quickly relieved by steroid administration (Lin et al., 2000b). The leukocytosis could resolve in all cases without chemotherapy (Camacho et al., 2000). Other mild side effects were encountered in about 40–50% of relapsed patients, such as fatigue, fever, edema, nausea, anorexia, diarrhea, emesis, headache, insomnia, cough, dyspnea, dermatitis, tachycardia, pain, hypokalemia, hypomagnesemia, and hyperglycemia. The most common (>10%) Grade 3 or 4 adverse events were abdominal pain, epistaxis, dyspnea, hypoxia, bone pain, thrombocytopenia, neutropenia, hypokalemia, and hyperglycemia (Shen et al., 1997; Soignet et al., 1998; Cohen et al., 2001). Of note, evident polyneuropathy compatible with chronic arsenic toxicity was noted in a few patients who received As_2O_3 maintenance therapy and one even had marked distal muscular atrophy (Huang et al., 1998). Cardiac dysrhythmias were reported with arsenic use (Huang et al., 1999). In a recent report from Japan, prolonged QT intervals were observed in all patients during

induction therapy with As_2O_3 and ventricular premature contractions were noticed during eight of 12 courses of therapy (Ohnishi et al., 2000). QT prolongation was also seen in 16 (38%) patients in the study from FDA (Cohen et al., 2001), which could return to baseline following cessation of As_2O_3.

In a recent clinical study conducted in SIH (Niu et al., 1999), 11 newly diagnosed APL patients received As_2O_3 treatment with informed consent. The CR was obtained in eight (72.7%) of them. However, As_2O_3 treatment resulted in elevated plasma liver transaminase levels in seven cases and two died of severe hepatic toxicity, in contrast to the mild liver dysfunction in about one third of the patients treated for relapsed APL. It was thus recommended that ATRA should be used as the first line drug for remission induction in newly diagnosed APL cases, while As_2O_3 can be either included into multi-drug consolidation/maintenance clinical trials after the first CR or used as a rescue for relapsed cases.

Post-remission treatment among relapsed APL patients

In a report by SIH, 33 patients subject to distinct post-remission treatment protocols after arsenic-induced CR were followed-up for 8–48 months (Niu et al., 1999). Three groups were formed, including four cases treated with chemotherapy alone (group A), 18 with As_2O_3 alone (group B) and 11 with both As_2O_3 and chemotherapy (group C). Although the estimated disease-free survival (DSF) rates at 1 and 2 years in all patients were 63.6% and 41.6%, respectively, recurrence of leukemia developed in 3/4 cases in group A, 12/18 in group B and 2/11 in group C. Combining chemotherapy and As_2O_3 seems able to decrease the relapse and to yield a statistically significant better survival status compared to arsenic alone as post-remission treatment (Niu et al., 1999). Recent data showed that the estimated 2-year DFS (49.1%) was not different in the low dose As_2O_3 treatment group as compared with that of the standard dose (Shen et al., 2001).

Molecular remission

RT–PCR analysis in both newly diagnosed and relapsed groups showed that application of As_2O_3 for a short period of time was not sufficient to eliminate minimal residual disease. Long-term use of the drug could, nevertheless, lead to a molecular remission at least in some patients (Niu et al., 1999). Of five patients reported by Zhang et al. (2000), two achieved molecular remission (negative RT–PCR for PML–$RAR\alpha$ fusion transcripts) by the end of the second and third cycles of As_2O_3 therapy.

Dual activities of As_2O_3 on APL cells in vivo and in vitro: induction of apoptosis and differentiation

The clinical effectiveness of As_2O_3 in APL has stimulated research activities aiming at an understanding of its mechanisms of action. Although many questions remain to be understood, in vivo and in vitro data suggested that induction of apoptosis and partial differentiation of APL cells is likely to constitute the cellular basis of the effects of As_2O_3 (Figure 1).

Evidences from APL patients

Initial clinical observations showed that during daily continuous intravenous infusion of As_2O_3 in APL, a large amount of myelocyte-like cells and degenerative, dying cells with condensed nuclei losing cytoplasm appeared in both BM and peripheral blood, with the gradual reduction of leukemic promyelocytes. Interestingly, the myelocyte-like cells were derived from abnormal promyelocytes because they still carried the PML–$RAR\alpha$ gene as revealed by the fluorescence in situ hybridization (FISH) analysis. Those degenerative cells were positively labeled by in situ terminal deoxynucleotidyl transferase, which is characteristic of programmed cell death (Chen et al., 1997). The presence of leukocytosis and RAS-like syndrome during arsenic treatment also supports the idea that As_2O_3 could induce differentiation. A similar finding was described by Huang et al. (1998). Soignet et al. (1998) performed a further in vivo analysis on the mechanisms of action of As_2O_3. They reported that As_2O_3 therapy induced a progressive decrease in the proportion of cells expressing CD33, an antigen associated with primitive myeloid cells, along with an increase in the proportion of cells expressing CD11b, an antigen restricted to mature myeloid elements. As_2O_3 also induced the increment of cells that simultaneously expressed both antigens and displayed a hybrid signal of PML and $RAR\alpha$ genes on FISH analysis. Interestingly, serial Western blot analysis of BM mononuclear cells during As_2O_3 treatment showed induced expression of the precursor forms of caspase 2 and caspase 3 and activation of both caspase 1 and caspase 3.

Evidences from APL models and in vitro studies

Using syngenic grafts of leukemic blasts from PML–$RAR\alpha$ transgenic mice, Lallemand-Breitenbach et al. (1999) demonstrated that As_2O_3 induced apoptosis and modest differentiation of APL cells in vivo, and significantly prolonged the survival of the diseased animals. In subcutaneous tumors which were formed by implantation of ATRA-resistant UF-1 APL cells into hGM-CSF-producing transgenic SCID mice (Kinjo et al., 2000), tumor size was decreased to half the initial one after As_2O_3 treatment, with the appearance of cells showing typical apoptotic morphology. Meanwhile, one of the As_2O_3-treated mice showed mature granulocytes in the diminished tumor.

In order to approach the complex mechanisms, in vitro effects of pharmacologic concentrations of As_2O_3 on fresh APL cells and APL cell lines including ATRA-sensitive NB4 cells, and ATRA-resistant NB4-derived sublines MR2, R1 and R2, were studied. The initial study showed that As_2O_3 at $1.0–2.0\ \mu M$ could sig-
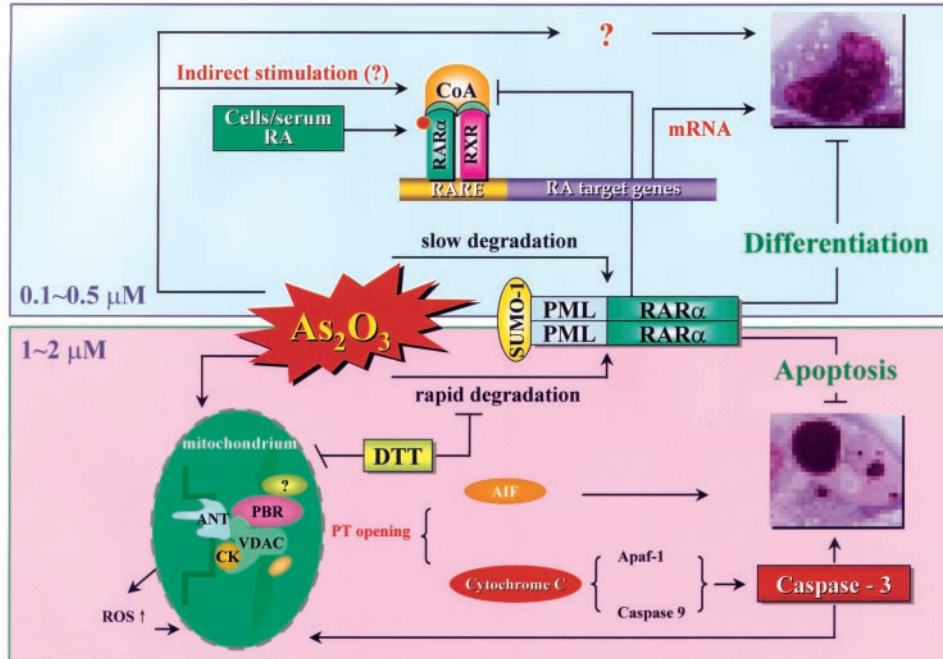
Figure 1 Working hypothesis for the mechanism of action of As$_2$O$_3$ in remission induction of APL. As$_2$O$_3$ induces remission of APL patients via two independent pathways: i.e., by triggering partial differentiation and inducing apoptosis. High-dose As$_2$O$_3$-induced apoptosis involves mainly the -SH groups-related ΔΨm collapse due to the opening of the PT pore, which leads to the release of pre-apoptotic factors from mitochondria to cytoplasma, followed by caspase activation and degradation of specific substrates. On the other hand, low-dose As$_2$O$_3$-induced differentiation might be related to cyclic AMP pathway, nuclear receptor activity and/or regulation of histone acetylation. As$_2$O$_3$ at both high- and low-dose triggers the degradation of PML–RARα. PML–RARα oncoprotein may therefore represent the common target for both ATRA and As$_2$O$_3$. However, the underlying mechanism seems to be different, the former targeting RARα through specific binding whereas the latter targeting PML moiety through SUMO-1-conjugation in a yet unknown manner. →: Stimulation. ⊣: Inhibition

nificantly induce apoptosis in APL cells regardless of their sensitivity to ATRA (Chen *et al*., 1996, 1997), which was widely confirmed by other groups (Kitamura *et al*., 1997, and see later). In order to establish an *in vitro* model of differentiation, NB4 cells were treated with 0.1–0.25 μM of As$_2$O$_3$ over a long time of culture. Indeed, after 6–9 days of treatment, cells started to present morphologic differentiation with CD11b expression and differentiation-associated cytochemical features. The differentiation seems only a partial one since most cells were blocked at the myelocyte to metamyelocyte stage of maturation and no significant increase of nitroblue-tetrazolium (NBT) reduction, a classical marker of granulocytic maturation, was observed in NB4 cells (Chen *et al*., 1997). Later, Gianni *et al*. (1998) isolated an As$_2$O$_3$-resistant NB4 subline (NB4-AsR). These cells failed to undergo apoptosis, but maintained the partial differentiation response to arsenic. Of note, ATRA-triggered differentiation and apoptosis were accelerated in As$_2$O$_3$-treated NB4-AsR cells.

Mechanisms of As$_2$O$_3$-induced apoptosis

A relatively wide spectrum of As$_2$O$_3$-induced apoptosis

Several groups reported that HL-60 and U937 cell lines, both are of myeloid lineage, had weak responses to

1 μM As$_2$O$_3$ in terms of growth and survival inhibition (Chen *et al*., 1996; Kitamura *et al*., 1997; Zhang *et al*., 1998), but a few groups observed that As$_2$O$_3$ could induce apoptosis in these cells (Wang *et al*., 1998). Although different culture situations and/or variations of cell lines might be responsible for such a discrepancy (McCabe *et al*., 2000), it may suggest that the cell type specificity of As$_2$O$_3$-induced apoptosis is limited. Indeed, over the past few years, several studies found that As$_2$O$_3$ could also inhibit cell growth and/or induce apoptosis among some lymphoma and solid tumor cell lines in a dose-dependent way. As$_2$O$_3$ could also cause apoptosis in proliferating layers of human umbilical vein endothelial cells and prevent capillary tubule and branch formation under *in vivo* and *in vitro* assay conditions (Roboz *et al*., 2000), raising the possibility of inhibiting metastasis. However, it should be noted that significant induction of apoptosis in cell systems other than APL may sometimes require high concentration As$_2$O$_3$ (5–10 μM), a dose difficult to be achieved *in vivo*.

Mitochondria are involved in As$_2$O$_3$-induced apoptosis

The fact that As$_2$O$_3$ induces apoptosis in a variety of cell types suggests that some common mechanisms should be involved. It is well established that mitochondria play a major rate-limiting role in apoptosis. Various pro-apoptotic signals converge on

the mitochondria and trigger progressive disruption of mitochondrial transmembrane potentials ($\Delta\Psi$m) by modulating the permeability transition pore complex (PTPC), a multiprotein complex which interacts with the apoptosis-regulating protein Bcl-2/Bax family (Kroemer and de Thé, 1999; Costantini et al., 2000a). As do most chemotherapeutic drugs, As_2O_3 can also induce an early $\Delta\Psi$m collapse in intact APL cells (Cai et al., 2000), maligant lymphocytes (Zhu et al., 1999) and carcinoma cell lines (Shen et al., 1999; Seol et al., 2001). As_2O_3-induced nuclear apoptosis also required mitochondria in an in vitro cell-free system, and As_2O_3 acted on isolated mitochondria to induce PTPC opening and opened the purified, reconstituted MPT in vitro in a cyclosporin A- and Bcl-2-inhibitable fashion (Larochette et al., 1999). These data support that mitochondria, PTPC in particular, are important target of As_2O_3. Besides, in the APL model, glutathione (GSH)-reducing agent dithiothreitol (DTT) could effectively block As_2O_3-induced $\Delta\Psi$m collapse and apoptosis. In contrast, buthionine sulfoximine (BSO), a depleter of cellular GSH, and ascorbic acid substantially enhanced the effect of As_2O_3 (Zhu et al., 1999; Dai et al., 1999; Jing et al., 1999; Cai et al., 2000). BSO could also make As_2O_3-resistant HL60 and U937 cells to become sensitive to the compound. Therefore, it is presumed that As_2O_3-induced $\Delta\Psi$m collapse and apoptosis are associated with dithiol oxidation or cross-linking, which has been shown to be associated with a higher probability of PTPC opening (Figure 1, lower part). Initially, we speculated that adenine nucleotide translocator (ANT) may be a target of As_2O_3 (Cai et al., 2000), since it is an important component of PTPC and contains the vicinal thiol group. In addition, ANT alone reconstituted into artificial lipid bilayers suffices to confer a membrane permeabilization response to thiol cross-linking agents. However, a recent report showed that As_2O_3 could not cause the oxidation of a critical cysteine residue (Cys^{56}) of purified ANT (Costantini et al., 2000a). In addition to the direct effects on PTPC, As_2O_3 could disrupt mitochondria through some indirect mechanisms, such as the induction of reactive oxygen species (ROS). Nevertheless, there are controversies in literature with regard to the role of ROS. It was reported that the increased intracellular peroxide levels were accompanied by As_2O_3-induced apoptosis, which could be inhibited significantly by N-Acetyl-L-cysteine (a thiol-containing antioxidant), diphenylene iodonium (an inhibitor of NADPH oxidase), 4,5-dihydro-1,3-benzene disulfonic acid (a selective scavenger of O_2^-) and catalase (Chen et al., 1998). However, other data showed that DTT blocked apoptosis induced by As_2O_3, but it could not antagonize As_2O_3-induced ROS production (Zhu et al., 1999). In the experiments with butylated hydroxyanisole, Uslu et al. (2000) suggested that the apoptotic activity of As_2O_3 in prostate and ovarian cancer cells was not through inducing superoxide generation.

Next to PTPC opening is the release of pro-apoptotic factors from mitochondria into the cytosol, where they activate sequentially upstream and downstream caspases such as caspase-9 (Costantini et al., 2000b; Brenner and Kroemer, 2000). Indeed, As_2O_3 could induce the release of cytochrome c from mitochondria to the cytosol (Chen et al., 1998; Jing et al., 1999). The activation of caspase-3 and cleavage of its specific substrates such as poly (ADP-ribose) polymerase (PARP) could also be seen in most cells treated with As_2O_3 (Akao et al., 1999; Huang et al., 2000; Cai et al., 2000; Jing et al., 1999) (Figure 1, lower part). In addition to caspase-3, Kitamura et al. (2000a) showed that caspase 8 and Bid were also activated by As_2O_3 in a GSH concentration-dependent manner in NB4 cells. An inhibitor of caspase 8 blocked not only the activation of caspase 3 but also the loss of $\Delta\Psi$m. However, Sternsdorf et al. (1999) realized that As_2O_3-induced apoptosis was caspase 3 independent and was only partially blocked by a global caspase inhibitor. In experiments conducted by SIH, caspase-3 was activated in 2 μM As_2O_3-treated Namalwa and Raji cells, but not in 1 μM of As_2O_3-treated BJAB, MOLT-4 and SKW-3 cells despite significant apoptosis, indicating that caspase-3 was not always necessarily involved in As_2O_3-induced apoptosis (Zhu et al., 1999).

Apart from the effect on mitochondria, as a pleiotropic agent, As_2O_3 may have additional actions that also contribute to the induction of cell death, such as the activation of c-Jun NH_2-terminal kinases (Huang et al., 1999) and inactivation of the GTP binding site of monomeric tubulin (Li and Broom, 1999). Although As_2O_3 does not increase p53 level or activity in most cells, a marked increase in p53 protein level was seen during As_2O_3-induced apoptosis in a human gastric cancer cell line, while co-incubation with p53 anti-sense oligo-nucleotide suppressed both p53 over-expression and apoptosis induced by As_2O_3 (Jiang et al., 2001). Hence, mechanisms of action of As_2O_3 may be very complex, depending on dose, cell type, or cellular environment (McCabe et al., 2000).

Mechanisms of As_2O_3 induced-differentiation of APL

As previously mentioned, during the $0.08 - 0.16$ mg/kg/day i.v. As_2O_3 treatment, plasma As_2O_3 levels were basically in the range of $0.1 - 0.5$ μM, corresponding to the concentrations inducing primarily differentiation of APL cells in vitro (Chen et al., 1996, 1997; Shen et al., 1997, 2001). However, the mechanism underlying As_2O_3-induced differentiation is still poorly understood. The fact that arsenic-resistant NB4-AsR subline failed to undergo apoptosis, but maintained the partial differentiation response to As_2O_3 suggested that distinct pathways may be responsible for the differentiation and apoptosis (Gianni et al., 1998). Recently, we showed that 0.1 μM As_2O_3 did not induce differentiation in ATRA-resistant APL cell lines. Furthermore, low-dose arsenic could induce partial differentiation of ATRA-sensitive, but not ATRA-resistant, HL-60 cells. In addition, the gene expression patterns modified by low-dose As_2O_3 overlapped with

those by physiological concentrations of ATRA (Cai *et al.*, 2000). These data suggested a possible cross-talk between the retinoid signaling and the regulatory pathways modulated by As_2O_3 (Figure 1, upper part). Of note, *in vivo* studies proposed that As_2O_3 and ATRA generate synergistic effect, such as an accelerated tumor regression and prolonged survival of animals (Lallemand-Breitenbach *et al.*, 1999; Kinjo *et al.*, 2000, Rego *et al.*, 2000), although the benefit of their combined use in cell culture is controversial (Chen *et al.*, 1996, Gianni *et al.*, 1998; Shao *et al.*, 1998; Jing *et al.*, 2001). Nevertheless, As_2O_3 showed no direct effect on RA receptors, in that the interaction of wild-type RARα and PML – RARα with nuclear receptor co-repressor was not affected, and arsenic showed no obvious effect on the transactivation activity of RARα in an RARE-luciferase reporter system (Chen *et al.*, 2001 and unpublished data). Interestingly, as will be discussed below, the differentiation of APL cells seems to be closely related to the modulation of PML – RARα, the key protein in leukemogenesis.

Modulation of PML and PML – RARα

The unique response of APL to As_2O_3 implies the importance of intrinsic properties of APL cells. Interestingly, both wild-type PML and PML-RARα fusion protein have been shown to be major targets of arsenic treatment. PML is a tumor suppressor involved in complex functions including growth arrest and apoptosis induction. Normally PML is located in the nucleus on a specific subdomain named PML nuclear body (NB). In APL cells, due to the heterodimerization of PML – RARα with wild-type PML, PML – NBs are disrupted into nuclear microspeckle pattern, with loss of PML functions (Melnick and Licht, 2000, Andre *et al.*, 1996). A surprising finding was that $0.1 – 2.0 \mu M$ of As_2O_3 targets PML and PML – RARα onto NBs, followed by degradation of these proteins (Chen *et al.*, 1996, 1997; Zhu *et al.*, 1997; Sternsdorf *et al.*, 1999; Gianni and de Thé, 1999). Furthermore, As_2O_3 increases covalent modification of PML by SUMO-1, a small ubiquitin-like protein. The SUMO-1-polymodified forms of PML are compartmentalized exclusively in the PML – NBs in non-APL cells, while the unmodified form of PML is found in the soluble nucleoplasmic fraction (Muller *et al.*, 1998; Sternsdorf *et al.*, 1999). Therefore, As_2O_3-enhanced PML sumoylation constitutes the basis of the restoration and accumulation of the PML in enlarged NBs in As_2O_3-treated APL cells (for more information, please refer to the review article by Dr H de Thé in the same issue) (Figure 1).

There are controversies on the role of modulation of PML/PML – RARα proteins in As_2O_3-induced apoptosis in APL cells. Through analysis of U937 cells stably expressing exogenous *PML – RARα* gene, Sternsdorf *et al.* (1999) reported that As_2O_3 could induce apoptosis only in U937 cells expressing the *PML – RARα* but not in cells expressing *PLZF – RARα*, a fusion gene generated as a result of the variant translocation t(11;17)(q23;q21) in APL. However, degradation of PML – RARα and change of the PML-subcellular localization were similarly induced by As_2O_3 in NB4 cells and NB4-derived As_2O_3-resistant subline (Kitamura *et al.*, 2000a). Therefore, the contribution of PML – RARα and PML modulation to apoptosis under the arsenic treatment seems to be relative even if present. In contrast, the degradation of PML – RARα may play a crucial role in the differentiation of APL cells, since this protein modulation can occur in the presence of very low concentration $(0.1 – 0.25 \mu M)$ of As_2O_3, while arsenic induced neither differentiation of APL cells with t(11;17) nor degradation of PLZF – RARα protein (Koken *et al.*, 1999; Kitamura *et al.*, 2000b). The low-dose arsenic shows marked cell type selectivity, since it causes maturation of APL cells but exhibits little effect on other cancer cells, although a recent report suggested that low-dose As_2O_3 can increase the lysis of myeloma cells by lymphokine-activated killer cells (Deaglio *et al.*, 2001). Importantly, there is also a marvelous target selectivity of As_2O_3, in that only the PML – RARα, but not the wild-type RARα, is degraded. The relief of the transcriptional repression of PML – RARα may pave the way for the operation of physiological RA signaling in granulocytic differentiation, particularly at the stage of promyelocytes (Figure 1, upper part). The possible synergistic effect of the arsenic with a number of important regulatory pathways such as RAR/RXR (Cai *et al.*, 2000), Cyclic AMP (Zhang JW, Zhu Q, Tong JH, unpublished data) and histone deacetylase inhibitor may also enhance the restoration of the cell differentiation program.

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