Performance Status (ECOG 基準)

Grade	定義
0	全く問題なく活動できる．発病前と同じ日常生活が制限なく行える
1	肉体的に激しい活動は制限されるが，歩行可能であり，軽作業や座っての作業は行うことができる．例：軽い家事，事務作業
2	歩行可能で自分の身の回りのことはすべて可能だが作業はできない．日中の50％以上はベッド外で過ごす．
3	限られた自分の身の回りのことしかできない．日中の50％以上をベッドか椅子で過ごす．
4	全く動けない．自分の身の回りのことは全くできない．完全にベッドか椅子で過ごす．

ECOG = Eastern Cooperative Oncology Group

この基準は全身状態の指標であり，局所症状で活動性が制限されている場合は，臨床的に判断する

急送一次報告書

【FAX 送信先】中皮腫臨床試験センター
FAX：0798-45-6783 （TEL：0798-45-6088）

（急送報告に該当する有害事象発生から 72 時間以内に上記 FAX 番号へ送信して下さい）

報告日	(西暦)　　年　　月　　日
施設名 / 担当医名	
記入者署名	
連絡先	FAX　　－　　－　　TEL　　－　　－

(1) 症例に関する情報

登録番号	

(2) 有害事象の分類

有害事象発生日	(西暦)　　年　　月　　日
分類	□ プロトコール治療中もしくは治療中止・終了後 30 日以内のすべての死亡 □ 治療に関連して発生した予期されない Grade4 の非血液毒性 □ その他

有害事象の概要（有害事象の具体的内容，関連する検査データ，因果関係に関する考察などを含む）

(3) 有害事象と因果関係が疑われる治療

□ 薬物療法　□ 放射線治療　□ 外科的治療　□ その他の治療

(4) プロトコール治療との因果関係についての報告者の評価

□ 明確に関連あり(definite)　□ 恐らく関連あり(possible)
□ 関連あるかもしれない(possible)　□ ありそうにない(unlikely)
□ 関係ない(not related)　□ 評価不能

中皮腫臨床試験センター記入欄

処理日 : (西暦)　　年　　月　　日　　研究事務局署名:

□ 急送二次報告　　□ 通常報告

急送報告または通常報告に該当する有害事象発生から 7 日以内に
中皮腫臨床試験センターまで FAX(0798-45-6783)または郵送してください

報告日	(西暦)　　年　　月　　日
施設名 / 担当医名	
記入者署名	
連絡先	FAX　　－　　－　　TEL　　－　　－

(1) 症例に関する情報

登録番号	

(2) 有害事象の分類

有害事象発生日	(西暦)　　年　　月　　日
分類	□ 死亡:最終治療日より　⇒　□30日以内　□31日以降 □ Grade 4の非血液毒性　⇒　□予期されないもの　□予期されるもの □ 予期されない Grade 2 or 3 の有害事象 □ その他

有害事象の内容とプロトコール治療との因果関係

(3) 有害事象と因果関係が疑われる治療

□ 薬物療法　□ 放射線治療　□ 外科的治療　□ その他の治療

(4) プロトコール治療との因果関係についての報告者の評価

□ 明確に関連あり(definite)　□ 恐らく関連あり(possible)
□ 関連あるかもしれない(possible)　□ ありそうにない(unlikely)
□ 関係ない(not related)　□ 評価不能

(5) 症例報告の詳細 (別紙添付 ___ 枚)

別紙(A4 版書式自由)に記載して, 本報告書に添付すること

中皮腫臨床試験センター記入欄

処理日 ：（西暦）　　年　　月　　日　　研究事務局署名：

Ⅲ．研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書　籍　名	出版社名	出版地	出版年	ページ
中野孝司	悪性胸膜中皮腫症例	中川和彦、伊藤良則、朴　成和、古瀬純司、直江知樹	研修医必携　症例から学ぶメディカルオンコロジー改定４版	医薬ジャーナル社	大阪市	2011	73-84
中野孝司	胸膜中皮腫（メソテリオーマ）	西條長宏、加藤治文	インフォームドコンセントのための図解シリーズ	医薬ジャーナル社	大阪市	2011	34-37
中野孝司	胸膜腫瘍	山口　徹、北原光男、福井次矢	今日の治療指針	医学書院	東京都	2012	304-306
中野孝司	胸膜中皮腫	社団法人日本呼吸器学会	DVDで学ぶ実践呼吸器病学No. 5		東京都	2012	5-9
中野孝司	胸膜中皮腫	技術情報協会	がん領域の希少疾患の医療ニーズ　希少疾患/難病の診断・治療と製品開発	技術情報協会	東京都	2012	686-696
中野孝司	中皮腫	日本臨床腫瘍学会	新臨床腫瘍学改定第3版	南江堂	東京都	2012	360-363
福岡和也	第VI章　代表的な胸部病変の種類と特徴.	日本肺癌学会集団検診委員会胸部X線写真による肺癌検診小委員会	肺がん検診のための胸部X線読影テキスト	金原出版株式会社	東京都	2012	202-218
田端千春	中皮細胞の生理機能	中野孝司	胸膜全書―胸膜疾患のグローバルスタンダードー	医薬ジャーナル社	大阪市	2013	39-40
中野孝司 栗林康造	中皮細胞傷害と修復・再生機序	中野孝司	胸膜全書―胸膜疾患のグローバルスタンダードー	医薬ジャーナル社	大阪市	2013	53-59
水野鉄也、横井香平	全身麻酔科下胸腔鏡検査、VATS	中野孝司	胸膜全書―胸膜疾患のグローバルスタンダードー	医薬ジャーナル社	大阪市	2013	67-71
福岡和也	びまん性悪性胸膜中皮腫	中野孝司	胸膜全書―胸膜疾患のグローバルスタンダードー	医薬ジャーナル社	大阪市	2013	254-261

渋谷景子	強度変調放射線治療	中野孝司	胸膜全書ー胸膜疾患のグローバルスタンダードー	医薬ジャーナル社	大阪市	2013	312-316
岡田守人	胸膜肺全摘術	中野孝司	胸膜全書ー胸膜疾患のグローバルスタンダードー	医薬ジャーナル社	大阪市	2013	325-329
長谷川誠紀	胸膜切除/肺剥皮術	中野孝司	胸膜全書ー胸膜疾患のグローバルスタンダードー	医薬ジャーナル社	大阪市	2013	334-339
田中文啓	悪性胸膜中皮腫に対する外科治療を含む集学的治療	中野孝司	胸膜全書ー胸膜疾患のグローバルスタンダードー	医薬ジャーナル社	大阪市	2013	342-351
中野孝司	悪性胸膜中皮腫の増加と治療戦	貫和敏博、杉山幸比古、門田淳一	呼吸器疾患最新の治療	日本臨床腫瘍学会	東京都	2013	72-76
中野孝司	悪性中皮腫マーカー(可溶型メソテリン関連ペプチド)	和田攻、大久保明行、矢崎義雄、大内尉義	臨床検査ガイド	文光堂	東京都	2013	933-935
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中野孝司	胸膜疾患	小川　聡	内科学書(改定第8版)	中山書店	東京都	2013	435-441
中野孝司	珪肺、石綿肺、胸膜中皮腫		スーパービジュアル呼吸器疾患	成美堂出版	東京都	2013	218-219 220-222 288-292
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中野孝司	転移性肺腫瘍	山口　徹、北原光男	今日の治療指針2014年度版	医学書院	東京都	2014	308-309
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発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
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Yamada S, Tabata C, Fukuoka K, Nakano T. et al	Clinical significance of pleural effusion mesothelin in malignant pleural mesothelioma	Clin Chem Lab Med	49	1721-1726	2011
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Ayuko Sato, Hasegawa S, Nakano T. et al	Establishment of a cell line from a Japanese patient useful for generating and in vivo model of malignant pleural mesothelioma	Cancer Sci	102	648-655	2011
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Eguchi R, Kubo S, Tabata C, Nakano T, et al	Deficiency of Fyn protein is prerequisite for apoptosis induced by Src family kinase inhibitors in human mesothelioma cells	Carcinogenesis	33	969-975	2012
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Nogi Y, Nakano T, Tabata C, Fukuoka K, et al.	AMP converted from intracellularly transported adenosine upregulates p53 expression to induce malignant pleural mesothelioma cell apoptosis	Cell Physiol Biochem	30	61-74	2012
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Hasegawa S	Early Mesothelioma Revisited.	Int J Clin Oncol	17	30-32	2012
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Tsujimura T, Fukuoka K, Hasegawa S, Nakano T, et al	Pathological and molecular biological approaches to diagnosis of early mesothelioma.	Int J Clin Oncol	17	40-47	2012
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Matuo M, Yokoi K, et al.	Inspiratory capacity as a preoperative assessment of patient undergoing thoracic surgery	Interact Cardiovasc Thorac Surg	14	560-564	2012
Ishiguro F, Yokoi K, et al.	Activated leukocyte cell-adhesion molecule (ALCAM) promotes malignant phenotypes of maliganant mesothelioma	J Thorac Oncol	7	890-899	2012
Elshazley M, Yokoi K, et al.	The circadian clock gene BMAL1 is a novel therapeutic target for malignant pleural mesothelioma	Int J Cancer	131	2820-2831	2012

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Tsubokawa N, Okada M, et al	Laparoscopic thoracic duct clipping for persistent chylothorax after extrapleural pneumonectomy	Ann Thorac Surg	93	e131-132	2012
Okada M, et al.	Radical hybrid video-assisted thoracic segmentectomy: long-term results of minimally invasive anatomical sublobar resection for treating lung cancer	Interact Cardiovasc Thorac Surg	14	5-11	2012
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Shimokawa M, Hasegawa S, Fukuoka K, Okada M, Yokoi K, Tanaka F, Yamanaka T, Daimon T, Nakano T.	A feasibility study of induction pemetrexed plus cisplatin followed by pleurectomy/decortication aimed at macroscopic complete resection for malignant pleural mesothelioma	Jpn J Clin Oncol	43	575-578	2013

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Yoneda K, Tanaka F, Fukuoka K, Tabata C, Nakano T, Hasegawa S, et al.	Circulating Tumor Cells (CTCs) in Malignant Pleural Mesothelioma (MPM)	Ann Surg Oncol		Epub ahead of print	2013
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Kawaguchi K, Yokoi K, et al.	FDG PET/CT is useful for detecting infiltration to the port sites in patients with malignant pleural mesothelioma	Gen Thorac Cardiovasc Surg	62	157-162	2014
Mimae T, Okada M, et al.	Increased ectodomain shedding of lung epithelial cell adhesion molecule 1 as a cause of increased alveolar cell apoptosis in emphysema.	Thorax	69	223-231	2014
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中野孝司	胸膜中皮腫の診断と治療の現状.	医療	65	22-25	2011
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Ⅳ．研究成果の刊行に関する一覧表

Journal of Cellular Physiology

Arsenic Trioxide Induces Apoptosis Through JNK and ERK in Human Mesothelioma Cells

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Malignant mesothelioma is an aggressive tumor of serosal surfaces, which is refractory to current treatment options. Arsenic trioxide (As_2O_3) is used clinically to treat acute promyelocytic leukemia, and also to inhibit proliferation of several solid tumors including hepatoma, esophageal, and gastric cancer in vitro. Here we found that As_2O_3 inhibited cell viability of a mesothelioma cell line, NCI-H2052. As_2O_3 induced apoptosis of NCI-H2052 cells, which was accompanied by activation of c-Jun NH_2-terminal kinase (JNK) 1/2, extracellular signal-regulated kinase (ERK) 1/2, and caspase-3. zVAD-fmk, a broad-spectrum caspase inhibitor, inhibited As_2O_3-induced apoptosis and activation of caspase-3, but not that of JNK1/2 and ERK1/2. Small interfering RNAs (siRNAs) targeting JNK1/2 suppressed As_2O_3-induced caspase-3 activation and apoptosis, indicating that JNK1/2 regulate As_2O_3-induced apoptosis though caspase cascade. Furthermore, JNK1 siRNA abrogated As_2O_3-induced JNK2 phosphorylation and JNK2 siRNA abrogated As_2O_3-induced JNK1 phosphorylation, suggesting that JNK1 and JNK2 interact with each other. Moreover, JNK1 siRNA, but not JNK2 siRNA, abrogated As_2O_3-induced ERK1/2 phosphorylation. JNK2 siRNA together with PD98059, a specific MAPK/ERK kinase inhibitor, suppressed As_2O_3-induced apoptosis more significantly than JNK2 siRNA alone. These results indicated that As_2O_3 induces apoptosis of NCI-H2052 cells mainly through JNK1/2 activation, and that ERK1/2 is involved in As_2O_3-induced apoptosis when JNK1/2 are inactivated.
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Malignant mesothelioma is an aggressive tumor arising from mesothelial cells of serous membranes including pleura, peritoneum, and pericardium (Robinson et al., 2005; Vogelzang, 2008; Tsao et al., 2009). Malignant mesothelioma is highly resistant to most chemotherapeutic agents (Vogelzang, 2008) and radiation, and surgical therapy generally show limited efficacy (Goudar, 2005; Vogelzang, 2008). So far combinations of pemetrexed and cisplatin appear to be the best chemotherapy regimen for malignant mesothelioma although the median survival was 12 months (Vogelzang et al., 2003). Malignant mesothelioma still remains an uncurable disease.

Arsenic trioxide (As_2O_3) is an ancient drug used in traditional Chinese medicine, which has been introduced to Western medicine in the last century (Antman, 2001). This drug has attracted wide interest for its ability to induce complete remission in patients with acute promyelocytic leukemia (APL), through induction of apoptosis and differentiation (Chen et al., 1997; Shao et al., 1998). Apoptotic effect of As_2O_3 is also observed in other cancer cells in vitro, including neuroblastoma (Akao et al., 1999), ovarian cancer (Du and Ho, 2001), gastric cancer (Zhang et al., 1999), esophageal cancer (Shen et al., 1999), and hepatoma (Oketani et al., 2002). However, the effect of As_2O_3 on mesothelioma cells has not been examined.

Induction of apoptosis usually depends on a common executor, caspase-3 (Kiechle and Zhang, 2002), which also cleaves and inactivates poly (ADP-ribose) polymerase (PARP), a DNA repairing enzyme (Decker and Muller, 2002). In As_2O_3-induced cell death, activation and involvement of caspase-3 has been reported in APL cells (Shao et al., 1998). The mitogen-actiated protein kinase (MAPK) pathways constitute a large kinase network that regulates a variety of physiological processes, including cell growth, differentiation, and apoptosis (Zhang and Liu, 2002). To date, three MAPK pathways have been characterized in detail: c-Jun NH_2-terminal kinase (JNK), p38 and extracellular signal-regulated kinase (ERK). Generally, JNK and p38 are key mediators of stress and inflammation responses evoked by a variety of physical, chemical, and biological stress stimuli. The ERK pathway is mostly induced by growth factors and mediates cell proliferation, but it also mediates apoptosis by stress stimuli (Lee et al., 2003). JNK has two ubiquitously expressed isoforms, JNK1 and JNK2, and a tissue-specific isoform, JNK3. MAPKs have been reported to be involved in As_2O_3-induced apoptosis of APL cells and other tumor cells (Ramos et al., 2006; Kang and Lee, 2008), with the specific MAPK pathway involved depending on the cell type and stimulus.

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In this study, we investigated cytotoxic and apoptotic effects of As_2O_3 on human mesothelioma cells. As_2O_3 inhibited the proliferation of mesothelioma NCI-H2052 cells and induced apoptosis of the cells at clinically feasible concentrations. Further clarification of cell signaling pathways revealed the involvement of JNK-dependent caspase cascade and ERK-dependent pathway in As_2O_3-induced apoptosis.

Materials and Methods

Cell culture and reagents

Human mesothelioma cell line NCI-H2052 was obtained from the American Type Culture Collection (Rockville, MD). Cells were cultured as monolayers in RPMI-1640 medium (Sigma, St. Louis, MO) with 10% fetal bovine serum (Sigma) under a humidified atmosphere containing 5% CO_2 at 37°C. zVAD(OMe)-fmk (zVAD) and PD98059 were purchased from BIOMOL (Plymouth Meeting, PA). All other chemicals were purchased from Sigma unless otherwise stated.

Treatment with As_2O_3 and inhibitors

Cells (2×10^5) were seeded in a 100 mm-dish (Becton Dickinson Labware, Franklin Lakes, NJ) and cultured for 24 h. Old medium was aspirated, and fresh medium containing As_2O_3 or vehicle (phosphate-buffered saline) was added together with zVAD, PD98059, or vehicle (dimethylsulfoxide) for inhibition treatment. Cells were then cultured for 2–72 h.

Cell viability analysis

Cells were seeded in a 96-well plate (Becton Dickinson Labware) at 2×10^3 per well. Cell viability was determined by a colorimetric assay using Cell Counting Kit-8 (CCK-8; Dojin Chemical Institute, Kumamoto, Japan). Briefly, after As_2O_3 treatments, WST-8, 2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2*H*-tetrazolium, monosodium salt was added in each well. The intensity of color developed was quantified using a micro plate reader (SPECTRAmax PLUS384, Molecular Devices, Sunnyvale, CA) to obtain the number of viable cells (Miyamoto et al., 2002). Color intensity was quantified by a microplate reader (SPECTRAmax PLUS384). Experiments were repeated in quadruplicate.

Flow cytometric analysis of apoptosis

Apoptosis was analyzed by flow cytometry using an Annexin V (Ax)-FITC Kit (Medical & Biological Laboratories Co. Ltd, Nagoya, Japan) according to the manufacturer's protocol (Vermes et al., 1995; van Engeland et al., 1996; de Cupis et al., 2003). Briefly, NCI-H2052 cells (1×10^5) treated with As_2O_3 were trypsinized, washed and then labeled with Ax-FITC and propidium iodide (PI). The fluorescence intensity was measured using a Cytomics FC 500

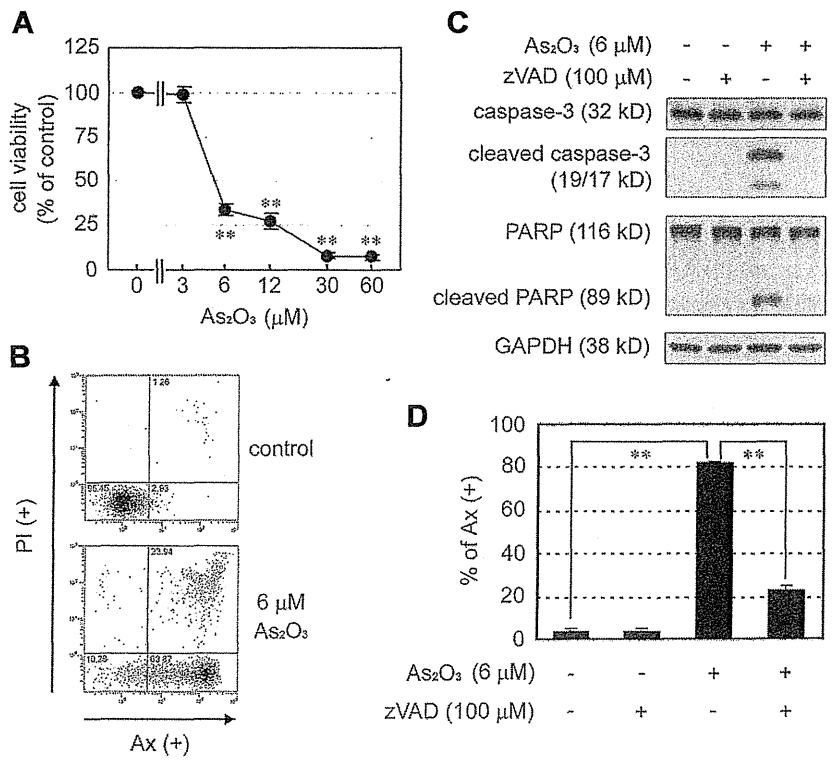

Fig. 1. As_2O_3 induces apoptosis of mesothelioma cells. A: As_2O_3 inhibits proliferation of human mesothelioma cell line. Human mesothelioma NCI-H2052 cells were treated with As_2O_3 for 72 h at the indicated concentrations. Cell proliferation was assessed using a colorimetric CCK-8 assay as described in Materials and Methods Section. B: Flow cytometric analysis using double staining with Ax and PI in NCI-H2052 cells. The numbers of early apoptotic cells (Ax+/PI−) and late apoptotic and necrotic cells (Ax+/PI+) were significantly increased 72 h after the treatment with 6 μM As_2O_3. C: As_2O_3 activates caspase-3, which was inhibited by zVAD(OMe)-fmk (zVAD). Cell extracts were prepared from NCI-H2052 cells treated with 6 μM As_2O_3 alone or together with 100 μM zVAD for 24 h. D: As_2O_3-induced apoptosis of NCI-H2052 cells is suppressed by zVAD. NCI-H2052 cells were treated with 6 μM As_2O_3 alone or together with 100 μM zVAD for 72 h, and analyzed for Ax(+) apoptotic cells by flow cytometry. The experiments in B and C were performed three times and the representative data are shown.