L-13 In vivo Anti-metastatic Action of Ginseng Protopanaxadiol saponins is Based on Their Intestinal Bacterial Metabolites After Oral Administration

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Ginseng (the root of *Panax ginseng* C. A. MEYER, Araliaceae) has been used for traditional medicine in China, Korea, Japan and other Asian countries for the treatment of various diseases including psychiatric and neurologic diseases as well as diabetes mellitus. So far, ginseng saponins (ginsenosides) have been regarded as the principal components responsible for the pharmacological activities of ginseng. Ginsenosides are glycosides containing an aglycone (protopanaxadiol or protopanaxatriol) with a dammarane skeleton and have been shown to possess various biological activities including the enhancement of cholesterol biosynthesis, stimulation of serum protein synthesis, immuno- modulatory effects and anti-inflammatory activity. Several studies using ginsenosides have also reported anti-tumor effects, particularly the inhibition of tumor-induced angiogenesis, tumor invasion and metastasis, and the control of phenotypic expression and differentiation of tumor cells.

Previously, it was reported that protopanaxadiol-type ginsenisides such as Rb1, Rb2 and Rc are metabolized by intestinal bacteria after oral administration to their final derivative 20-*O*-β-D-glucopyranosyl-20(*S*)-protopanaxadiol, which is referred to as M1 or compound K. In the present study, we investigated *in vivo* and *in vitro* anti-metastatic activities of M1 in comparison with ginsenosides Rb_1, Rb_2 and Rc and its inhibitory mechanism of action.

We found that protopanxadiol-type ginsenosides and their major metabolite M1 markedly inhibited the lung metastasis of B16-BL6 melanoma cells when they were administered p.o. (Fig. 1). In contrast, three consecutive i.v. administration of M1 after tumor inoculation resulted in a significant inhibition of lung metastasis, whereas ginsenosides did not show any inhibitory effect. Only M1 showed the inhibitiory effect on the proliferation, migration and invasion of tumor cells *in vitro*, but ginsenosides did not affect the activities. Pharmacokinetic study after oral administration of Rb_1 revealed that M1 was detected in serum for 24 h by HPLC analysis but Rb_1 was not detected. These findings suggest that the main bacterial metabolite M1 is an active component of orally administered ginsenosides, and that the anti-metastatic effect by oral administration of ginsenosides may be primarily mediated through the inhibition of tumor invasion, migration and growth of tumor cells by their metabolite M1.

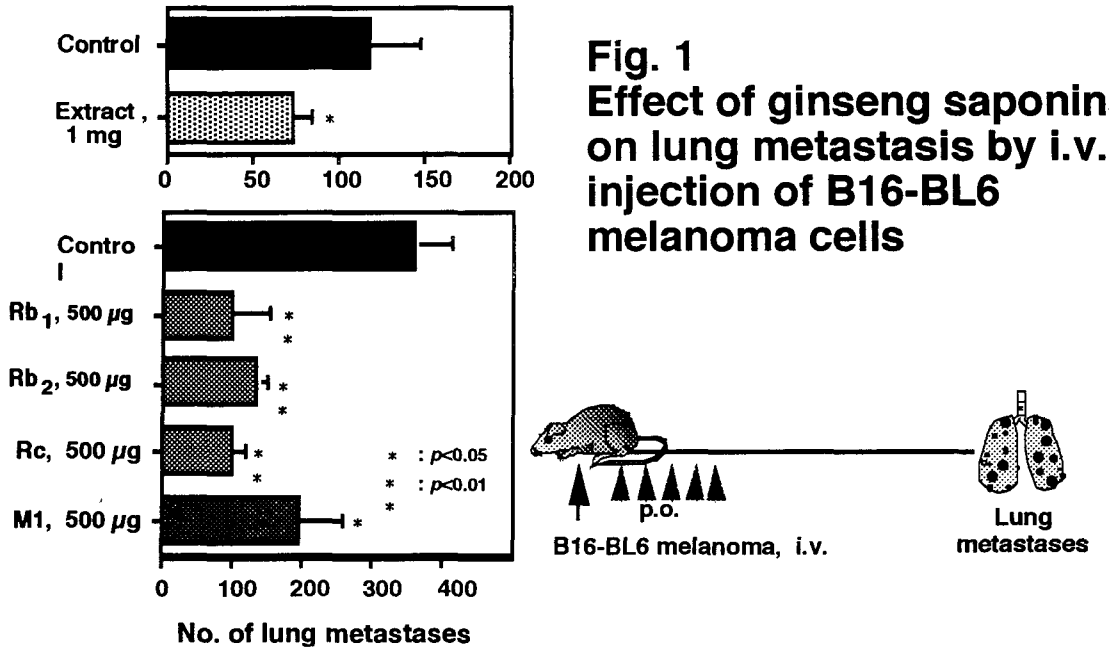
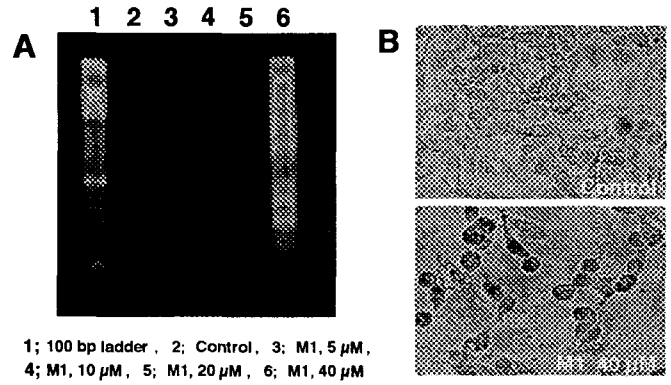


Fig. 1
Effect of ginseng saponins on lung metastasis by i.v. injection of B16-BL6 melanoma cells

However, the detail of how the active metabolite M1 affects the growth of tumor cells has not been clear. We therefore examined the inhibitory mechanism of M1 on the proliferation of tumor cells *in vitro*. M1 showed relatively selective cytotoxicity against B16-BL6 murine melanoma and HT-1080 human fibrosarcoma cells compared with that of normal fibroblasts in a time- and concentration-dependent manner. In contrast, the incubation with ginsenoside-Rb_1 did not affect the morphology of tumor cells or cell proliferation. M1-treated cells induced apoptotic cell death with a swelling-shape morphology. Actually, treatment with M1 (40 μM) induced the ladder fragmentation of the extracted DNA, *i.e.* apoptosis (Fig. 2).

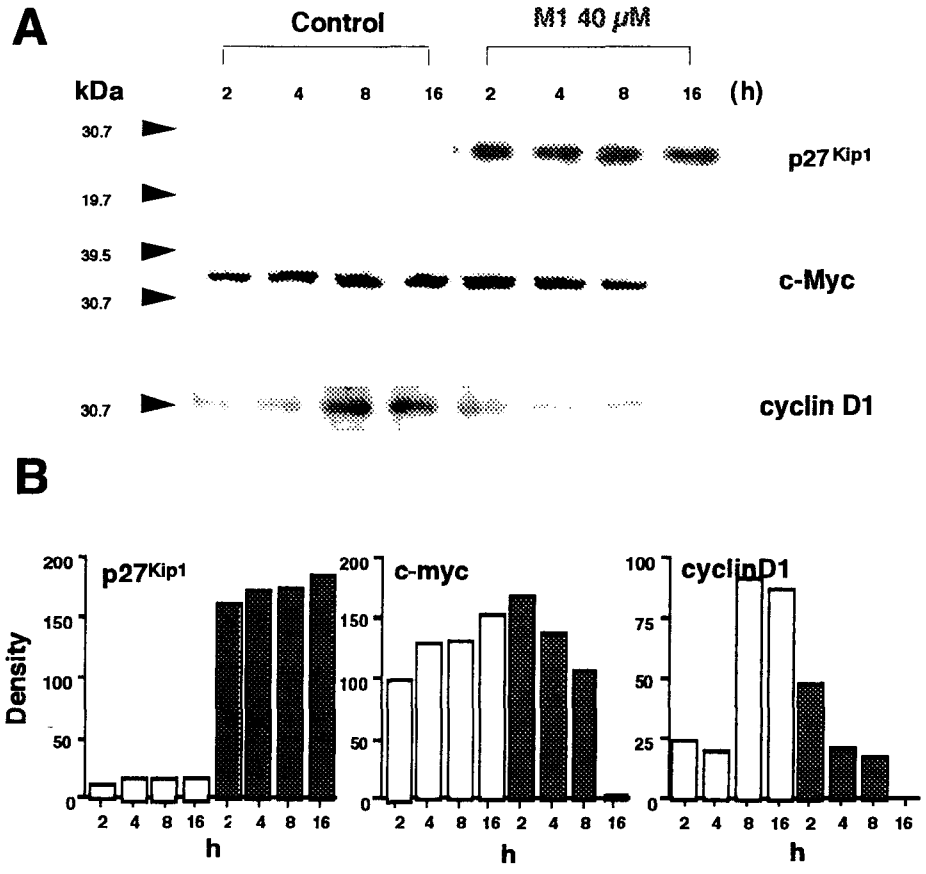
Fig. 2 M1-induced DNA fragmentation of B16-BL6 cells and the cell morphology



Although the molecular events that drive the apoptotic signaling pathway are not entirely clear, some apoptosis-related proteins such as cyclin D1, c-Myc or cyclin-dependent kinase (CDK)

inhibitors have been reported to be associated with cell division and proliferation. M1 treatment (40 μM) markedly increased the expression of p27Kip1, which is known to inhibit the CDK activity, as compared with the untreated control. On the other hand, a proto-oncogene product c-Myc as well as cyclin D1 have been reported to be overexpressed in the proliferative phase of various types of tumor cells. The expression of c-Myc and cyclin D1 was down-regulated by M1 treatment in a time-dependent manner. Thus, M1 might cause the cell-cycle arrest in tumor cells through the up/down-regulation of these cell-growth related molecules, and consequently induce apoptosis. The possibility that M1 inhibits or promotes these apoptosis-related molecules such as Bcl-2, Bax and caspases is under investigation.

Fig. 3 Western blot analysis of p27Kip1, c -Myc and cyclin D1 in B16-BL6 cells treated with M1



To examine the intra-cellular distribution of M1 after the incubation with tumor cells, we used dansyl M1. The fluorescent signal of dansyl M1 was detected in the cytosol and nuclei 15-min after incubation, and thereafter was observed predominantly in the nuclei. These findings suggest that the apoptotic cell death is induced by intra-cellular M1 through the transcriptional regulation of several cell-growth associated proteins. However, the regulatory mechanisms of M1 at the transcriptional level will be needed to investigated in detail.

In conclusion, the present study demonstrated that a metabolite of ginseng protopanaxadiol

saponin (M1), with anti-metastatic property, inhibited the proliferation of tumor cells in a time- and concentration-dependent manner, and in addition induced apoptotic cell death. The induction of apoptosis by M1 involved the up-regulation of the CDK-inhibtor p27Kip1 as well as the down-regulation of c-Myc and cyclin D1. The nucleosomal distribution of M1 suggests that the modification of these molecules is induced by transcriptional regulation.

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