Inhibitory Effect of Cytostatin on Spontaneous Lung Metastases of B16-BL6 Melanoma Cells

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Tumor cells may interact with extracellular matrices (ECM) such as laminin, fibronectin and collagen type IV, at a variety of stages in the metastatic cascade[1,2]. Some low molecular weight inhibitors of cell adhesion which reduce experimental and/or spontaneous lung metastases have been reported[3~5].

Recently, we have isolated a novel inhibitor of cell adhesion to components of ECM, named cytostatin from *Streptomyces* sp. MJ654-NF4 and we showed that it has inhibitory activity against experimental lung metastases of B16-F10 melanoma cells[6].

In this paper, we report the inhibitory effect of cytostatin on spontaneous metastases of B16-BL6 melanoma cells.

The spontaneous lung metastases were assessed as follows: B16-BL6 melanoma cells were maintained by serial cultures and 1×10^6 cells were inoculated into mice (BDF_1 female 5~6 weeks old) sc into the left hind footpad. Cytostatin was administered ip on various schedules before or after excision of primary tumor as indicated in Table 1. Primary tumors were surgically removed on day 20 and mice were sacrificed on day 16 after the excision. Lungs were removed and fixed with MeOH, and the metastatic foci were counted under a dissection microscope.

As shown in Table 1, cytostatin reduced the number of metastatic foci in comparison to untreated controls. The extent of the inhibition depended on the dose and the administration schedule. The most significant inhibition was obtained in the administration at 1.25 mg/kg for 19 days starting on day 1 or 12 days starting 8 days after tumor inoculation. The inhibitory ratios were 72.9% in 19 days and 68.4% in 12 days, respectively. Both schedules were completed before excision of primary tumor. Since the administration of cytostatin on either schedule could not significantly inhibit primary tumor growth in footpad, it can be considered that the effect might be due to inhibition of tumor cell adhesion to ECM. Furthermore, a significant inhibitory effect was obtained at 1.25 mg/kg/day for 2 weeks after the excision. Those results indicate that cytostatin can be an antagonist of ECM receptors of tumor cells such as laminin and collagen type IV and can inhibit experimental or spontaneous tumor metastases.

The mechanisms of inhibitory action are now under study.

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Table 1. Inhibition of spontaneous lung metastases of B16-BL6 melanoma cells by cytostatin.

Dose (mg/kg ip)	Schedule (Day)	No. of mice	No. of metastatic foci Mean ± SD (range)	Inhibition (%)	t-test[1)]
0		9	72.8 ± 33.9 (39-143)	0	
1.25	1-19	7	19.7 ± 13.1 (4-41)	72.9	p<0.01
0.31	1-19	7	43.4 ± 32.4 (11-99)	40.3	n.s.
1.25	8-19	7	23.0 ± 18.3 (4-49)	68.4	p<0.01
0.31	8-19	7	46.7 ± 41.0 (15-93)	35.9	n.s.
1.25	21-35	7	38.3 ± 13.2 (23-51)	46.7	p<0.05
0.31	21-35	7	54.7 ± 33.8 (17-108)	24.8	n.s.

1) Compared with untreated control mice by Student's t-test.

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