Anticancer and Some Biological Activities of Thiazinotrienomycin B

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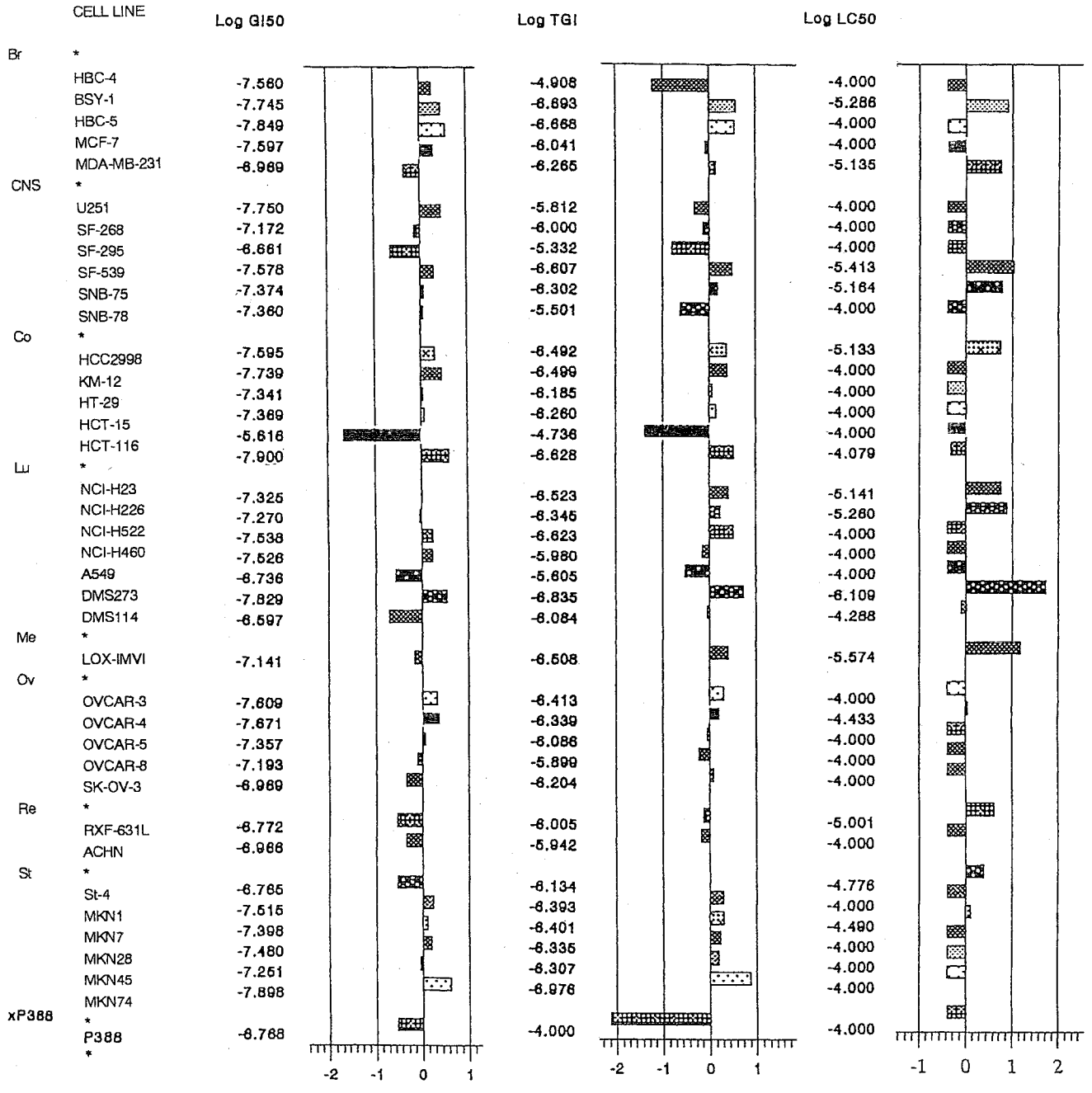
Cancers are caused by complex combinations of somatic mutations in genes controlling cell growth and differentiation. Due to wide phenotypic diversities of the disease, it is unlikely that a single drug will cure every type of cancers. As a primary screening system for new anticancer antibiotics, we have been using several cancer cell lines of human origins and selecting microbial products that are growth-inhibitory *in vitro* to certain cell lines, rather than to all the cell lines, to avoid compounds of non-specific toxicity. Thiazinotrienomycins were isolated for their activities somewhat specific to HeLa than to other cell lines which were available to us at the time of our investigation. Thiazinotrienomycin B (referred to as TT-B), a minor member in quantity, turned out to be the strongest in inhibiting growth *in vitro* of HeLa cells[1]. In the present paper, we report the pattern of differential growth inhibition *in vitro* by TT-B of a disease-oriented panel of human tumor cell lines[2] (the mean graph, Fig. 1) and the inhibition by TT-B of xenograft of BSY-1 (breast) in nude mice. Growth inhibition by TT-B against a panel of 38 human cancer cell lines. A human cell line panel combined with data base analysis was established by YAMORI *et al.*[3] Cell lines for the panel were not the same as those originally chosen by NCI, Bethesda[4], considering the high incidence of stomach cancers in Japan, for instance. Numbers of cell lines and their origins (organs) are as follows: 6 breast (Br), 6 central nervous system (CNS), 5 colon (Co), 7 lung (Lu), 1 melanoma (Me), 5 ovary (Ov), 2 kidney (Re), 5 stomach (St), and a mouse leukemia P388. The COMPARE[3,4] of the mean graph (Fig. 1) suggested that TT-B would neither be a DNA-binder, a DNA-breaker, nor a mitotic inhibitor, but have a unique mode of action.

Growth of BSY-1 xenograft was clearly inhibited by TT-B at doses of 50, 60, 72 and 86 mg/kg (Fig. 2). As described in the legend, TT-B was administered in a single shot, suggesting that TT-B killed the tumor cells (cytocidal) rather than temporarily inhibited their growth (cytostatic). Additional xenograft tests conducted with HT-29 (Co) and NCI-H460 (Lu) showed no or little effects at a dose of 72 mg/kg, however (data not shown). The positive (BSY-1) and negative (HT-29 and NCI-H460) results may reflect differences in sensitivities of these cell lines to TT-B, especially when LC_{50} values are compared; log LC_{50} values for BSY-1, HT29 and NCI-H460 were -7.724, ≥ -4.00 and ≥ -4.00, respectively (Fig. 1). Based on the LC_{50} values, BSY-1 is over 5,000 times more sensitive to TT-B than HT-29 or NCI-H460. LC_{50} values may predict sensitivities of different xenografts to the drug. Log LC_{50} values for SF-295 (CNS), DMS-273 (Lu) and LOX-IMVI (Me) were -5.413, -6.109 and -5.574, respectively, even smaller than that for BSY-1. We will soon conduct xenograft tests using these cell lines.

We reported recently that TT-B at low concentrations inhibited the function of EGF receptor, hence inhibited serum-dependent cell growth, in a highly sensitive cell line (SC-6, human stomach), which is not included in the cell line panel[2]. IC_{50}, TGI and LC_{50} of TT-B for SC-6 were -8.647, -7.419 and -6.771, respectively, though the assay conditions were not exactly identical to those in assays for the cell line panel[3]. Since the inhibition of EGF receptor is reversible, this effect alone dose not seem to cause cell-killing. We do not know whether xenograft of SC-6 will be inhibited, as was the case for BSY-1 by TT-B. Growth *in vitro* of BSY-1 was not so dependent on serum concentrations as was that of SC-6, suggesting that inhibition of EGF receptor is not involved in the cell-killing activity (LC_{50}) of TT-B against BSY-1. To know the mode of cell killing, additional cellular pharmacology studies are needed. It is tempting to presume that TT-B induces apoptosis at high concentrations in BSY-1. We will test this possibility.

Fig. 1. Growth inhibition by TT-B against a panel of 38 human cancer cell lines.



Growth inhibition (GI_{50}, 50% inhibition; TGI, 100% inhibition; and LC_{50}, 50% killing, *i.e.*, 150% inhibition) was determined and the results were analyzed and graphically displayed as reported[3,4].

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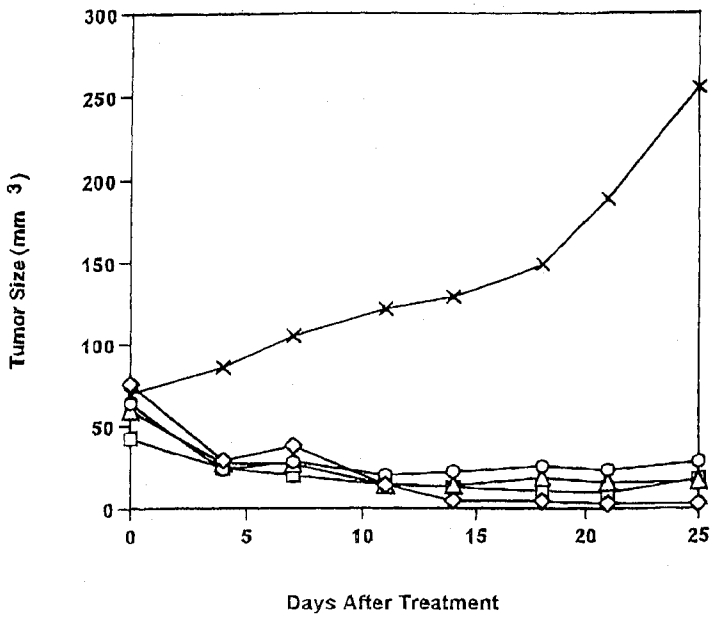
Fig. 2. Anticancer effect by TT-B on xenograft of BSY-1 (breast) in nude mice.

× Control, ◇ 86 mg/kg, ○ 72 mg/kg, △ 60 mg/kg, □ 50 mg/kg.



3×10^6 cells of BSY-1 were inoculated subcutaneously in the back of every mouse. 10 days later, the mice were inspected to carry solid tumors of 2~3 mm in diameter and appropriate mice were divided at random into groups of 3 mice. Each group received a single intravenous injection of TT-B solutions (Day 0), which had been prepared as below. Changes in the tumor size were followed at intervals of 2~4 days. Control alone, which received the vehicle, consisted of 6 mice. TT-B was dissolved in DMSO and the solution was mixed with an equal volume of Cremopho EL and diluted with 10 times or more volumes of saline to make solutions containing wanted amount of TT-B (final concentration of DMSO was less than 5% v/v).

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