Selective Cytotoxicity of Mycophenolic Acid against Transformed Cells

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The retinoblastoma tumor suppressor protein (pRB) plays a central role in mammalian cell cycle control and is inactivated during the development of a wide variety of human cancers[1]. Human papillomaviruses (HPV) are highly associated with human cervical cancers and encode E6 and E7 oncoproteins, which bind and inactivate the tumor suppressors p53 and pRB, respectively[2].

In order to search for antitumor substances with selective cytotoxicity against transformed cells, we established immortalized cell lines with pRB inactivated by HPV16 E7 oncoprotein. Primary rat glia cells obtained from Wistar rat (18-day embryo) cerebral cortex were transfected with plasmids containing a neomycin-resistant gene and HPV16 E7 or both E6 and E7 oncogenes (pSVneo-E7P and pSVneo-E6E7)[3] by the calcium phosphate method[4]. Drug-resistant colonies were selected by incubation for 3 weeks in the presence of

Fig. 1. Structure of mycophenolic acid.

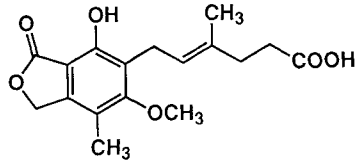
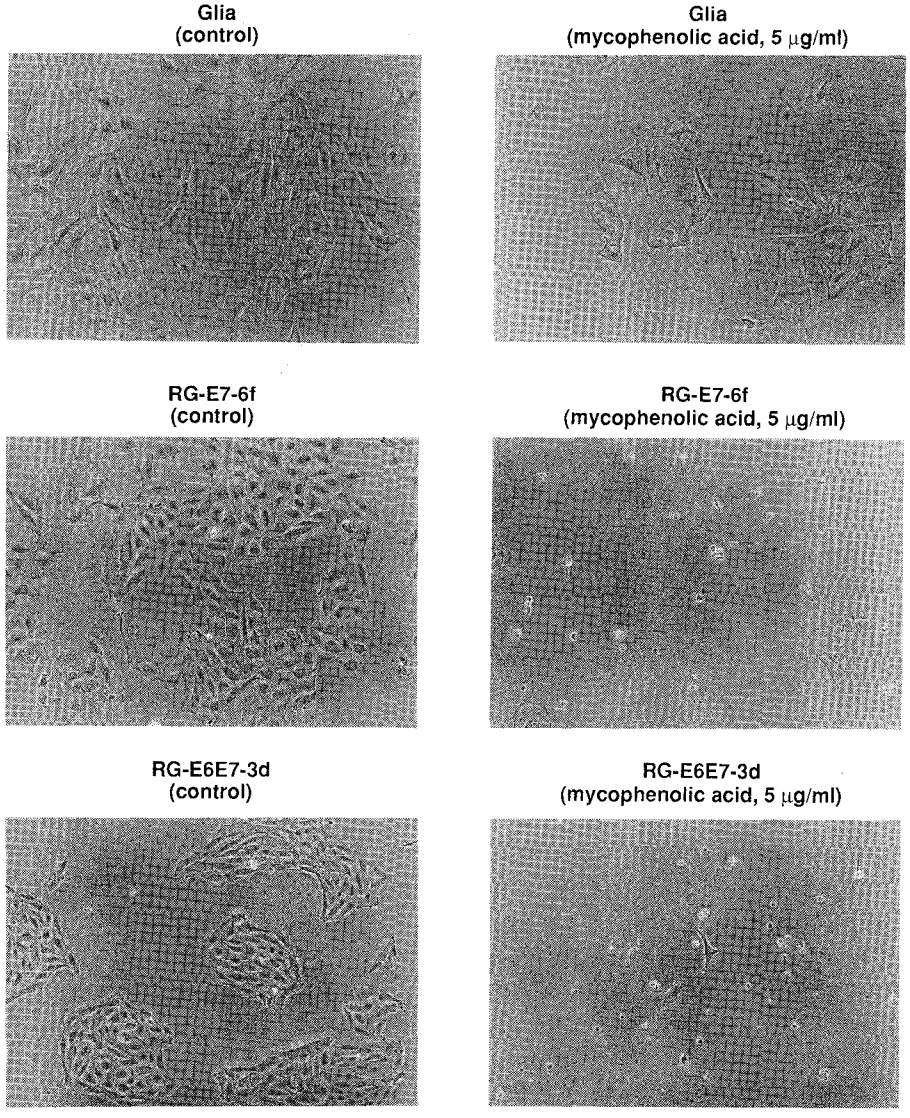
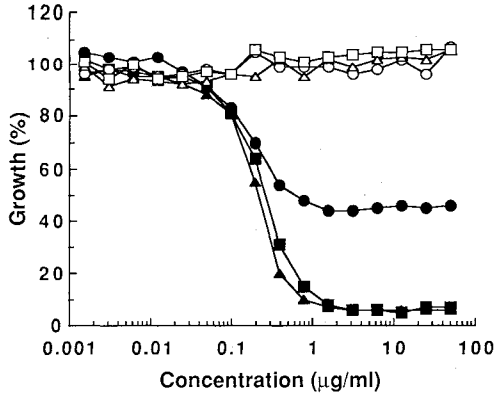


Fig. 2. Effect of MPA on the viability of normal and transformed rat glia cells.



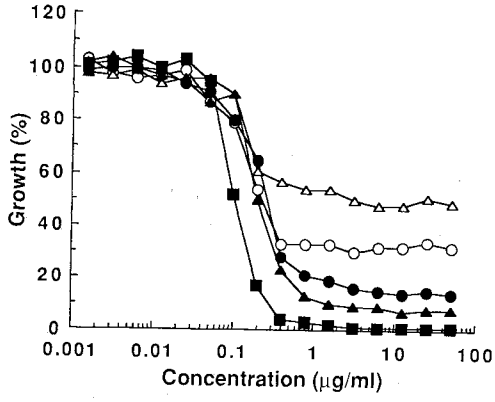
Cells were cultured for 72 hours in DULBECCO's modified EAGLE's medium supplemented with 10% fetal calf serum and 0.1% glucose in the presence or absence of 5 μg/ml of MPA.

Fig. 3. Effect of MPA on the growth of normal and transformed rat glia cells in the presence or absence of guanosine.



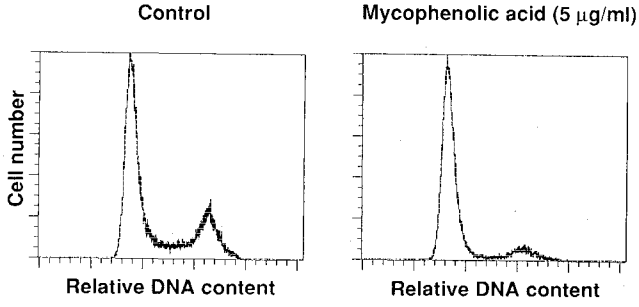
Cells were cultured with various concentrations of MPA for 72 hours in DULBECCO's modified EAGLE's medium supplemented with 10%d fetal calf serum and 0.1% glucose in the absence (●, ▲, ■) or presence (○, △, □) of 100 μg/ml of guanosine, and then the growth was measured by the MTT method. ●, ○ Glia cells; ▲, △ RG-E7-6f cells; ■, □ RG-E6E7-3d cells.

Fig. 4. Effect of MPA on the growth of normal and transformed rat 3Y1 fibroblasts.



Cells were cultured with various concentrations of MPA for 72 hours in DULBECCO's modified EAGLE's medium supplemented with 10% heat-inactivated fetal calf serum and 0.1% glucose, and then the growth was measured by the MTT method. ○ 3Y1, △ HR-3Y1, ● SR-3Y1, ▲ SV-3Y1, ■ Ad12-3Y1 cells.

Fig. 5. Flow cytometric cell cycle analysis of 3Y1 cells.



Cells in G1 phase, G2/M phase and S phase are represented by the first peak, the second peak and the area between the peaks, respectively.

400 μg/ml of G418, a neomycin analogue. Twelve weeks after transfection, immortalized cells were cloned by limiting dilution and designated RG-E7-6f and RG-E6E7-3d cell lines.

In the course of our screening for antitumor antibiotics by using these transformed cells, a fungal strain was found to produce an active substance, which was identified as mycophenolic acid (MPA) based on its physicochemical properties and NMR data. The structure of MPA is shown in Fig. 1.

MPA induced cell death against transformed glia cells (RG-E7-6f and RG-E6E7-3d). In contrast, MPA arrested the growth of normal glia cells without cytotoxicity. The viability of the cells treated with 5 μg/ml of MPA is illustrated in Fig. 2. Since MPA is known to be a potent inhibitor of inosinate dehydrogenase, a key enzyme of guanine nucleotide biosynthesis[6], the effect of MPA on the cell growth with or without guanosine was examined by formazan formation after treatment of the cells with 0.5 mg/ml of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). As shown in Fig. 3, 100 μg/ml of guanosine completely prevented the activity of MPA, suggesting that the cytotoxic and cytostatic effects of MPA are mediated *via* reduction of a guanine nucleotide pool.

The activity of MPA was further investigated using normal and transformed 3Y1 rat fibroblasts[7~9]. All 3Y1 cell lines were obtained from Japanese Cancer Research Resources Bank. The results are summarized in Fig. 4. MPA induced growth arrest against normal 3Y1 cells and v-H-*ras*-transformed cells (HR-3Y1), and caused cell death against 3Y1 cells transformed with simian virus 40 (SV-3Y1) and adenovirus type 12 (Ad12-3Y1). Cells transformed with v-*src* (SR-3Y1) exhibited an intermediate sensitivity to MPA-induced cytotoxicity.

Flow cytometric analysis on a Beckton Dickinson FACScan instrument revealed that MPA arrested the cell cycle of 3Y1 cells in G1 phase as shown in Fig. 5.

Cell lines highly sensitive to the killing effect of MPA commonly express viral oncoproteins including HPV E7, adenovirus E1A and simian virus 40 large T antigen, which can bind and inactivate pRB. Since inactivation of pRB by phosphorylation is required for progression from G1 into S phase, MPA seems to inhibit phosphorylation of pRB in normal cells. The inappropriate entry of pRB-inactivated cells into S phase under limiting concentration of dGTP might cause a DNA replication error and cell death.

MPA has been reported to reveal excellent antitumor activity *in vivo* and no toxicity in mice (LD_{50} >1,000 mg/kg, ip or po)[10]. In our assay system, other known antitumor antibiotics such as actinomycin D, adriamycin and mitomycin C showed cytotoxicity against normal glia cells and fibroblasts at their effective con-

centrations (data not shown). Therefore, this system is useful to search for antitumor agents with low toxicity. Further biological studies on MPA are in progress.

Acknowledgments

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