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PROPERTIES OF ADRIAMYCIN-RESISTANT CELL LINES ISOLATED FROM SYRIAN HAMSTER FIBROSARCOMA CELLS

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シリアンハムスター由来線維肉腫細胞より分離したアドリアマイシン耐性細胞株の性状

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We isolated three adriamycin (ADM)-resistant cell lines from a Syrian hamster fibrosarcoma cell line. These resistant tumor lines were 6 to 10 times more resistant to ADM than the parent line and exhibited cross-resistance to daunomycin, actinomycin D, chromomycin A_3 and vincristine, but not to mitomycin C, bleomycin, methotrexate or cycloheximide. Uptake and efflux studies with ^{3}H-ADM showed that one line incorporated and retained lesser amounts of ^{3}H-ADM than did the parent line, but the other two lines incorporated and retained as much of the drug as did the parent line. These results suggest the presence of a mechanism(s) of ADM resistance other than impaired accumulation of ADM due to amplified expression of multidrug resistance gene 1, in ADM-resistant lines.

シリアンハムスター由来線維肉腫細胞より3種類のadriamycin(ADM)耐性細胞株を分離した．これらの耐性株は，ADMに対して親株より6—10倍耐性であり，daunomycin, actinomycin D, chromomycin A_3 およびvincristineに対して交叉耐性を示したが，mitomycin C, bleomycin, methotrexateおよびcycloheximideに対しては交叉耐性を示さなかった．さらに，耐性株における^{3}H-ADMの取り込みと放出状態を親株と比較検討した．その結果，1株の耐性機構は，従来から報告されているように，ADMの取り込みの低下と放出の増大によるADMの細胞内蓄積低下によると考えられたが，他の2株では，ADMの取り込みおよび放出状態は親株と同様であり，交差耐性遺伝子1の増幅発現によるADMの細胞内蓄積低下だけでは説明できない新たな耐性機構の存在が示唆された．

The anthracycline antibiotic adriamycin (ADM) is commonly used as an antitumor agent, because it exhibits considerable cytotoxic activity against a broad spectrum of leukemias and solid tumors[1]-[3]. Tumor cells that are resistant to ADM frequently develop in the patients, but the cause of this resistance is not clear. Previous studies have shown that resistance to ADM in the ADM-resistant cell lines established from murine tumor cells is associated with decreased drug uptake[4]-[6], which may be related to alterations in the cell membrane[7]-[9] or may be the consequence of increased active efflux of the drug from the cells[10],[11].

Recently we established some ADM-resistant cell lines from Syrian hamster cells transformed by herpes simplex virus type 2 (HSV-2) and found unique resistant lines which incorporated and retained as much ADM

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as did the parent line[12]. To find out whether such lines can be isolated also from tumor cells which were passed *in vivo,* not only from the transformed cells which have been passaged serially *in vitro,* we established ADM-resistant lines from cultured cells of a hamster tumor produced by inoculating the transfomed cells. This report describes the isolation of such unique ADM-resistant lines from a tumor cell line.

The parent line 155-4-03T was derived from a fibrosarcoma that developed in a Syrian hamster inoculated subcutaneously with a clone (155-4-03) of HSV-2-transformed Syrian hamster cells (155-4)[13),(14)]. The methods for isolation of ADM-resistant lines were essentially the same as described previously[12]. Briefly, 155-4-03T cells were cultivated in the presence of 0.1 μg of ADM per ml (Kyowa Hakko Kogyo Co., Ltd., Tokyo) in growth medium [Eagle's minimum essential medium (MEM) supplemented with 10% fetal calf serum] for 2 days at 37°C and further cultivated in growth medium containing 0.25 μg of ADM per ml for 2 days at 37°C. Cells grown under the above conditions were seeded into 60-mm dishes (10^5 cells/dish) and incubated with growth medium containing ADM (0.25 μg/ml) at 37°C. At 23 days after seeding, cell colonies were isolated from several dishes and passaged serially in the medium containing ADM (0.25 μg/ml). In this study, three ADM-resistant lines (ADM^R-24, ADM^R-26 and ADM^R-30) were used between passages 15 and 28. During the passage, these resistant lines were exposed to ADM (0.25 μg/ml) continuously; however, the lines were always subcultured at least once in ADM-free medium before being used for tests.

Table 1. Cross-resistance of ADM-resistant cell lines to various drugs

Drug	IC_{50}(ng/ml)[a)]			
	155-4-03T	ADM^R-24	ADM^R-26	ADM^R-30
ADM	25	270(10.8)[b)]	180(7.2)	170(6.8)
Daunomycin	10	96(9.6)	84(9.4)	46(4.6)
Actinomycin D	0.8	10(12.5)	12(15.0)	6(7.5)
Chromomycin A_3	8	80(10.0)	80(10.0)	60(7.5)
Vincristine	15	210(14.0)	150(10.0)	51(3.4)
Mitomycin C	6	5(0.8)	8(1.3)	5(0.8)
Bleomycin	1,300	1,800(1.4)	2,000(1.5)	1,700(1.3)
Methotrexate	7	9(1.3)	8(1.1)	12(1.7)
Cycloheximide	56	80(1.4)	64(1.1)	52(0.9)

Cells were seeded in 60-mm dishes ($5\times10^2-1\times10^3$ cells/dish) containing 5 ml of growth medium with various drug doses (2 dishes/each drug dose) and incubated at 37°C for 10 days. Cell colonies were fixed, stained, and counted as described previously[12]. The efficiency of colony formation of the parent line and the three ADM resistant lines was 10 to 20%.

a) The IC_{50} value is the drug dose reducing the cell survival to 50% of the control (untreated culture).

b) The number in parentheses is the index of resistance which was calculated by dividing the IC_{50} value for the ADM resistant line by that for the parent line (155-4-03T) for each drug.

We determined the resistance of the parent and the three ADM-resistant tumor lines to various drugs with different functions by colony formation (Table 1). The resistant lines were 6 to 10 times more resistant to ADM than the parent line and exhibited cross-resistance to daunomycin (Meiji Seika Ltd., Tokyo), actinomycin D (Boehringer Mannheim, Mannheim, F. R. G.), chromomycin A_3 (Takeda Chemical Industries Ltd., Osaka) and vincristine (Shionogi Co., Ltd., Osaka), but not to mitomycin C (Sankyo Co., Ltd., Tokyo), bleomycin (Nippon Kayaku Co., Ltd. Tokyo), methotrexate (Takeda Chemical Industries Ltd.) or cycloheximide (Boehringer Mannheim).

The parent line and the three ADM-resistant lines were examined for uptake of ^{3}H-ADM (198 μCi/mg; Kyowa Hakko Kogyo Co., Ltd.). Cells were grown in ADM-free growth medium in 24-well microplates overnight at 37°C and washed once with MEM. Samples (500 μl, 0.05 μCi) of ^{3}H-ADM (0.5 μg/ml in MEM) were added to the wells (about 2×10^5 cells/well), and the plates were incubated at 37°C. At appropriate times, the cells were washed twice with phosphate buffered saline (PBS), harvested, and placed on Whatman GF/C filter paper disks (2 samples per time point) by means of an aspirator. The disks were washed 3 times with PBS and dried, and radioactivities were measured as described previously[12]. As shown in Fig. 1, line ADM^R-24 incorporated significantly smaller amounts of ^{3}H-ADM during 1 hr than did the parent line ($P < 0.01$). However, uptake of the drug by the other two lines, ADM^R-26 and ADM^R-30, was similar to that by the parent line, and there was no statistically significant difference between those resistant lines and the parent line.

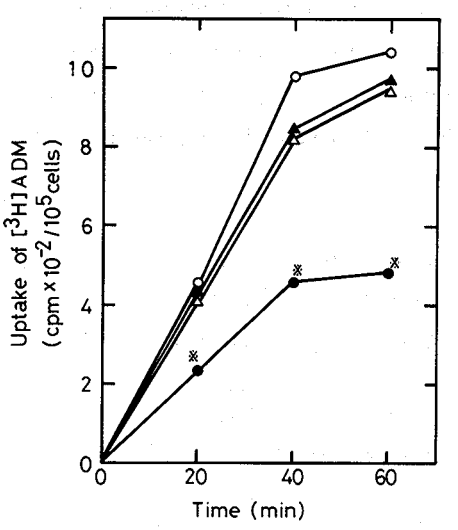

Fig. 1. Uptake of ^{3}H-ADM by the parent line and by the three ADM-resistant lines. ○, 155-4-03T (parent line); ●, ADM^R-24; △, ADM^R-26; ▲, ADM^R-30. ※ $P < 0.01$ (by Student's t test).

Efflux of ^{3}H-ADM from the parent line and from the three ADM-resistant lines was determined. The experiments were carried out as described previously[12]. Briefly, cells (1×10^7) were suspended in 5 ml of PBS containing 10 mM NaN_3 in test tubes, and samples of 20-μl (0.4 μCi) of ^{3}H-ADM (20 μg/ml in PBS containing 10 mM NaN_3) were added to the tubes. The cell suspensions were incubated for 30 min at 37°C, centrifuged, washed once with PBS, resuspended in 2 ml of PBS containing glucose (0.1%), and incubated at 37°C. At appropriate times, 150 μl samples of cell suspension were placed on the filter disks (2 samples per time point) and washed 3 times with cold PBS containing 10 mM NaN_3, by using an aspirator. The disks were dried, and radioactivities were measured. As shown in Fig. 2, line ADR^R-24 was better able to release ^{3}H-ADM than was the parent line ($P < 0.01$). However, the rates of efflux of the drug from lines ADM^R-26 and ADM^R-30 were similar to that from the parent line.

In this study, we isolated three ADM-resistant lines from a Syrian hamster fibrosarcoma cell line. The resistant tumor lines were 6 to 10 times more resistance to ADM than the parent line (155-4-03T cells). This

degree of ADM resistance of these resistant tumor lines was lower than that of ADM-resistant transformed lines which were 20 to 30 times more resistant to ADM than was the parent line (155-4-03 cells)[12]. Of the three ADM-resistant tumor lines, two lines (ADM^R-26 and ADM^R-30) exhibited neither decreased uptake of ADM nor increased efflux of the drug, indicating that such lines can be isolated from a tumor cell line passed *in vivo* as well as from the transformed line passaged serially only *in vitro*. The findings in this and previous[12] studies strongly suggest the presence of a mechanism(s) of ADM resistance other than enhanced outward transport of ADM due to overproduction of plasma membrane glycoprotein (P-glycoprotein) encoded by multidrug resistance gene 1[15]. Recently, Kramer *et al.* showed the presence of another mechamism that altered glutathione redox cycle, an important pathway in the detoxification of reactive oxygen, in ADM-resistant tumor cells[16].

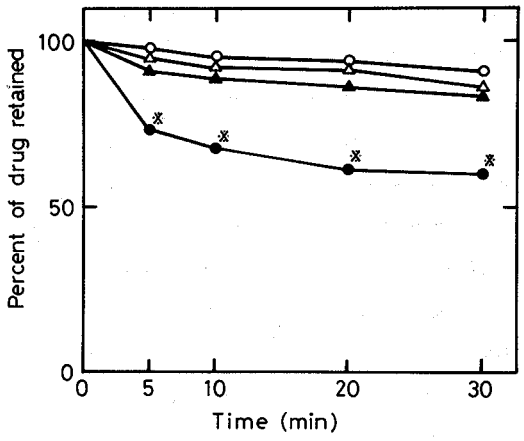

Fig. 2. Efflux of ^{3}H-ADM from the parent line and from the three ADM-resistant lines.
○, 155-4-03T (parent line); ●, ADM^R-24; △, ADM^R-26; ▲, ADM^R-30. ※ $P < 0.01$ (by Student's t test).

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