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Altered Expression of Epidermal Growth Factor Receptor Gene in a Classical Multidrug-resistant Variant of a Human Cancer Cell Line, KB

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A variant clone resistant to high doses of colchicine (KB-C1) derived from human cancer KB cell line is resistant to various anticancer agents. The KB-C1 cells were much more resistant to epidermal growth factor and a chimeric toxin, EGF-*Pseudomonas* exotoxin (PE), than the parental KB cells. KB-C1 cells have decreased numbers of EGF-receptors, though the affinity of the receptors is similar to that in the parental KB cells. A drug-sensitive revertant (C1-R2) partially recovered its EGF-receptor activity. Northern blot analysis showed a decreased level of EGF-receptor mRNA in KB-C1 cells, while the multidrug-resistance gene, *mdr-1*, was expressed at very high levels in KB-C1 cells, but not in KB or C1-R2 cells. The drug-resistant cells were less tumorigenic than the parental cells when injected into nude mice. A decreased expression of EGF-receptor in these cells may be one of the pleiotropic properties of multidrug-resistant cells and may perhaps represent the basis for their reduced tumorigenicity.

Key words: Multidrug resistance — Growth factor — Tumorigenicity

Cancer patients go into remission as a consequence of the effective action of anticancer agents, but if relapse occurs, the cancer may no longer respond to the same drugs. The cancer cells appearing in the patient after chemotherapy are often resistant to drug therapy, and drug-resistance in tumors is a serious clinical problem.[1] Chemotherapy appears to create a specific pressure that selects for resistant clones to become predominant in the tumor cell population. Acquisition of drug-resistant phenotype, pharmacological hideout of tumor cells and appearance of tumor cells arrested at the G_1/G_0 cell cycle state are supposed to be involved in the development of drug resistance during chemotherapy.[1]

Concerning the biochemical mechanism of simultaneous acquisition of resistance to many anticancer agents (multidrug-resistance; MDR), expression of a membrane glycoprotein (P-glycoprotein) with a molecular weight of 170,000 daltons is closely correlated with the membrane transport of drugs.[2-6] Over-expression of the MDR gene (*mdr*-1) is observed in resistant tomor cells,[7, 8] while experimentally selected MDR clones show enhanced efflux pump activity of anticancer agents possibly through the P-glycoprotein.[9]

Danks *et al.* reported that MDR cells derived from human leukemic cells also overexpressed other surface glycoproteins different from the P-glycoprotein.[10] Further study showed that expression of these resistance-associated glycoproteins might be related to differentiation or maturation of the leukemic cells.[11] Richert *et al.* reported that an MDR clone derived from human KB cells had decreased amounts of 72,000 and 75,000 dalton glycoproteins.[12] Ganglioside composition and glycoproteins were changed in these MDR cell lines.[13] One would expect that growth behavior might be changed during acquisition of multidrug-resistance since Biedler *et al.* showed that MDR cell lines were less tumorigenic than drug-sensitive parental cell lines.[14] Meyers *et al.* have reported that MDR cell lines isolated from some rodent cell lines have increased numbers of epidermal growth factor (EGF) receptors.[15] In this study, we examined whether an MDR clone of KB cell line shows aberrant response to growth factors, and whether altered response to a growth factor alters the tumorigenicity of the MDR cell line.

MATERIALS AND METHODS

Cell lines and cell culture KB is a subline isolated from human epidermoid carcinoma. KB-C1 (KB-Ch^R-8-5-11-14-24), a multidrug-resistant mutant, was selected by applying increasing concentrations of colchicine to KB cells.[16] This KB-C1 is the same clone as Ch^R-24 which we have used in our laboratory.[17, 18] C1-R2 is a cloned revertant of KB-C1 cells which was isolated after culturing KB-C1 cells in the absence of colchicine for 3 months. Cells were grown in monolayer in MEM (Nissui Seiyaku Co., Tokyo) containing 10% newborn calf serum (Microbiological Associates, Bethesda, MD), 1 mg/ml Bactopeptone (Difco Laboratories, Detroit, MI), 0.292 mg/ml glutamine, 100 μg/ml kanamycin and 100 units/ml penicillin.[17, 18]

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Drugs and chemicals EGF was obtained from Toyobo Co., Osaka. A toxic conjugate of *Pseudomonas* exotoxin (PE) and EGF (EGF-PE) was the generous gift from Dr. Ira Pastan (National Cancer Institute, NIH, Bethesda, MD).[19] ^{125}I-EGF (150–200 μCi/μg) was purchased from New England Nuclear, Boston, MA. Restriction enzymes were from Takara Co., Kyoto. A DNA labeling kit was purchased from Boehringer Mannheim. Nonidet P-40 (NP-40) (BDH Chemicals, Ltd., Poole, England) was obtained from Dai-ichi Chemical Co., Tokyo, and sodium dodecyl sulfate (SDS) was from Nacalai Tesque Inc., Kyoto.

Cellular sensitivity to EGF or EGF-PE assayed in terms of colony formation To determine cell survival, 300 cells were plated in 60 mm dishes and 16 h later, various doses of EGF or EGF-PE were added. After incubation for 10 days at 37°C, colonies were stained with 0.5% methylene blue in 50% ethanol and counted as described previously.[17, 18]

Scatchard analysis for ^{125}I-EGF binding The cells were plated at a density of 4×10^5 cell per 35 mm dish. After incubation for 24 h at 37°C, the medium was replaced with serum-free MEM containing 0.1 mg/ml bovine serum albumin and 10 m*M* HEPES, pH 7.5.[18, 19] The cells were cooled on ice for 15 min, then various concentrations of ^{125}I-EGF were added in the presence of a 200-fold higher dose of unlabeled EGF. After incubation for 2 h at 4°C, cells were washed twice with cold phosphate-buffered saline (PBS) and lysed with 1 *N* NaOH. The radioactivity was measured as ^{125}I-EGF binding activity and was analyzed according to the method described previously.[20]

Tumorigenicity assays Tumorigenicity of KB, KB-C1, and C1-R2 cells was assayed after subcutaneous inoculation of 2×10^6 or 2×10^7 cells into the flank of a mouse. Female Balb/c A/nu/nu/Jcl nude mice, 7 weeks of age, obtained from Clea Japan Inc., Tokyo, were used. Growth of tumors was regarded as positive if they were palpable 3 months after the inoculation. Tumor weight was determined for all palpable tumors, and ranged from 0.5 g to 3.5 g.

Northern blot analysis Cytoplasmic RNA was prepared as described[21] with a minor modification. Briefly, the cells were rinsed twice with cold PBS, scraped into PBS, and pelleted at 1000 rpm for 5 min. The cell pellet was resuspended in cell lysis buffer (1% NP-40, 5 m*M* NaCl, 5 m*M* Tris-HCl, pH 7.5, 0.5 m*M* $MgCl_2$) and centrifuged at 3000 rpm for 10 min. The supernatant was mixed with an equal volume of 1% SDS, 7 *M* urea, 0.3 *M* NaCl, 20 m*M* Tris-HCl, pH 7.5 and 2 m*M* EDTA. After serial extractions with phenol, phenol-chloroform and chloroform, RNA was precipitated with ethanol. Ten micrograms of RNA was fractionated on 1% agarose containing 2.2 *M* formaldehyde. After electrophoresis, the gel was stained with ethidium bromide, photographed and transferred to a nylon filter (Zeta probe, Bio-Rad) with 10×ssc (1.5 *M* NaCl, 150 m*M* sodium citrate, pH 7.0). The filter was prehybridized for 3 h at 50°C with buffer containing 5×ssc, 20 m*M* Na-phosphate (pH 7.0) 10× Denhardt's solution (1×Denhardt's solution=0.02% (w/v) each of bovine serum albumin, polyvinylpyrrolidone, and Ficoll), 7% SDS, and 100 μg/ml salmon sperm DNA. Hybridization was carried out for 16 h at 50°C in the same buffer containing 10% dextran sulfate. Filters were washed at 50°C twice in 3×ssc, 5% SDS, 10 m*M* Na-phosphate and 10×Denhardt's solution, and then twice in 1% SDS and 1×ssc. Autoradiography was carried out using Kodak XAR film.

Preparation of hybridization probe DNAs containing cDNA fragment from pE7 for EGF-receptor,[22] pHDR4.4 for *mdr-1*[23] and genomic fragment from Ha160 for β-actin[24] were digested by appropriate restriction enzymes. The cDNA fragments were purified from agarose gel and uniformly labeled with ^{32}P to a specific activity of 10^8–10^9 cpm/μg by primer-mediated DNA replication.[25]

RESULTS

Comparison of cellular sensitivity to EGF and EGF-PE of KB and KB-Cl cells We tested the effect of EGF on cultured MDR cells from clone KB-C1, and compared the results with those obtained for the parental drug-sensitive KB cells. The appearance of the two lines of cells differed markedly in the presence of EGF (Fig. 1). Most of the KB cells on a plastic dish were detached and rounded when 10 ng/ml of EGF was present (Fig. 1A and B), while no change in the morphology was observed in KB-C1 cells treated with EGF (Fig. 1C and D).

KB cells have EGF-receptors and their growth is affected by EGF. We examined the effect of EGF or EGF-PE on cell growth with a colony-forming assay. EGF was cytotoxic when added to KB cells at more than 0.1 ng/ml (Fig. 2). A striking difference was noted with the KB-C1 cells, where 50% inhibition of KB-C1 colony number was observed at more than 10 ng/ml, as compared with 0.2–0.3 ng/ml for KB cells. The 50% inhibitory dose (LD_{50}) of EGF for a revertant clone C1-R2 of KB-C1, which shows almost the same sensitivity to many anticancer agents as KB cells, was 0.3–0.4 ng/ml. Figure 3 shows the dose-response curves of KB, KB-C1 and C1-R2 to EGF-PE. The LD_{50} of EGF-PE was 90 ng/ml for both KB and C1-R2 cells, while that for KB-C1 cells was 800 ng/ml. These data show that the MDR clone KB-C1 has a higher resistance to cytotoxic doses of EGF or EGF-PE than the parental KB or the revertant C1-R2 cells.

Binding kinetics and expression of EGF receptor To determine the basis of the change in the cellular response

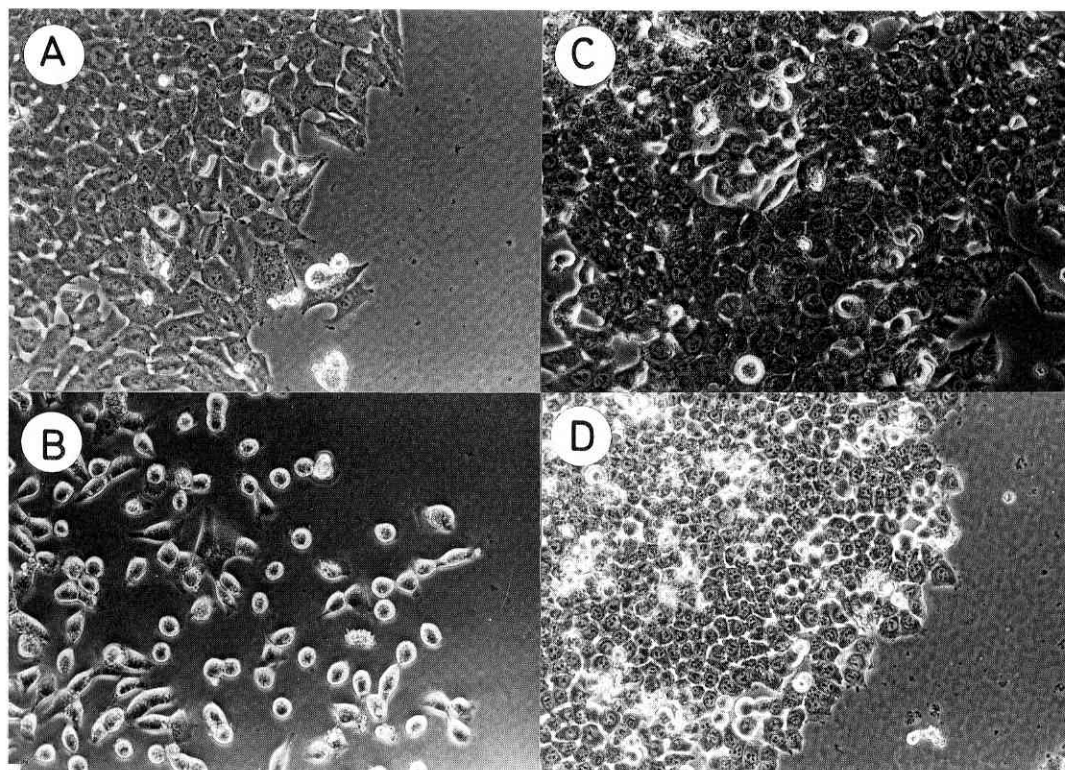

Fig. 1. Effect of EGF on the morphology of KB (A, B) and KB-C1 cells (C, D). Three hundred cells were inoculated per 60-mm plastic dish and the next day 10 ng/ml of EGF was added to one KB (B) and one KB-C1 (D) cell culture. Cell morphology 10 days thereafter is shown here. Untreated control cells of KB (A) and KB-C1 (C) are also shown.

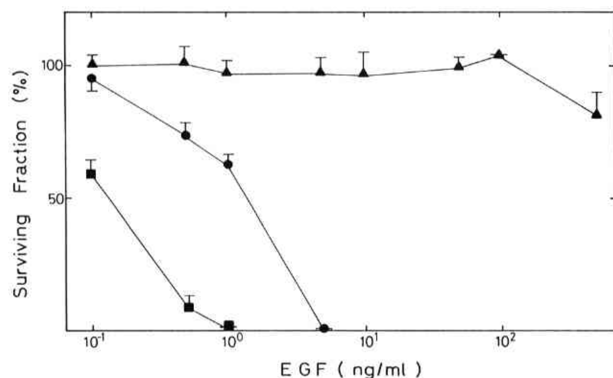

Fig. 2. Effect of EGF on cell survival, assayed in terms of colony-forming ability. Cells (300/60-mm dish) were plated and exposed to various concentrations of EGF for 9 days. KB (■), KB-C1 (▲) and C1-R2 (●). Points, mean of duplicate determinations; bars, SE.

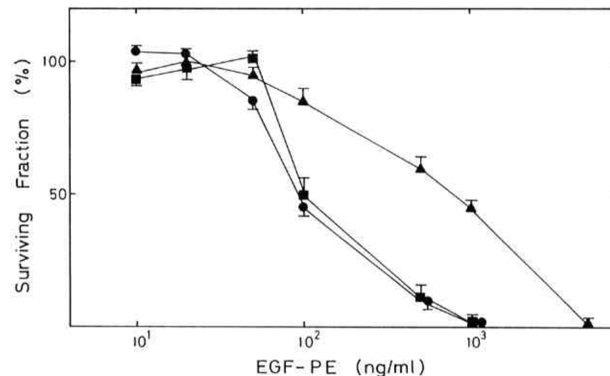

Fig. 3. Effect of EGF-PE on cell survival, assayed in terms of colony-forming ability. Cells (300/60-mm dish) were plated and exposed to various concentrations of EGF-PE for 9 days. KB (●), KB-C1 (▲) and C1-R2 (■). Points, mean of duplicate determinations, bars, SE.

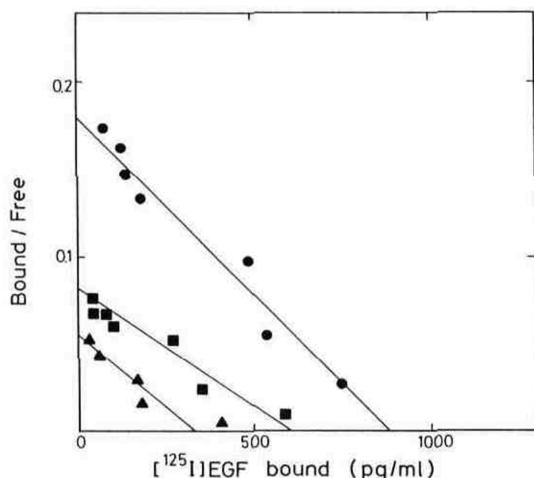

Fig. 4. Scatchard analysis of saturation kinetics for ^{125}I-EGF binding at 4°C to the cell surface of KB, KB-C1 and C1-R2 cells. KB (●), KB-C1 (▲) and C1-R2 (■).

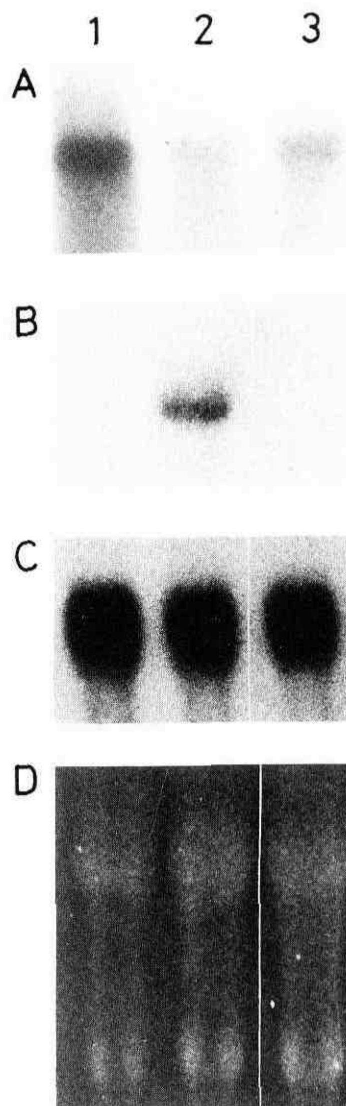

Fig. 5. Northern blot analysis for EGF receptor in KB, KB-C1 and C1-R2 cells. Ten μg of RNA from KB (lane 1), KB-C1 (lane 2) and C1-R2 (lane 3) cells was used for hybridization with cDNAs of EGF receptor (panel A), *mdr-1* (panel B) and β-actin (panel C). The bottom row indicates rRNA of 18S and 28S (panel D).

to EGF by the KB-Cl cells, we compared the EGF binding activities of each cell line by using various doses of ^{125}I-EGF. Scatchard analysis of the saturation kinetics for ^{125}I-EGF binding to the cell surface of KB, KB-C1 and C1-R2 showed 86,000, 32,000 and 60,000 receptors per cell, respectively (Fig. 4). The values of affinity (Kd) of the EGF receptors were estimated to be 1.4 nM (KB), 0.9 nM (KB-C1) and 1.2 nM (C1-R2), respectively. To determine the cellular expression of the EGF-receptor, northern blot analysis was carried out. As seen in Fig. 5, an MDR-related gene, *mdr-1,* is highly expressed in KB-C1, but not in KB and C1-R2 cell. Expression of the β-actin gene was similar in all three cell lines. By contrast, the cellular level of EGF-receptor mRNA in KB-C1 was less than one-half that of KB or C1-R2. Binding activity for EGF-receptor (Fig. 4) and northern blot analysis (Fig. 5) suggested that expression of the receptor mRNA is decreased in the MDR clone in comparison with its parental cell line. The reduction in mRNA levels suggests that synthesis of the receptor is reduced.

Comparison of tumorigenicity of KB-C1 and KB cells We examined the tumorigenicity of each cell line by inoculation into nude mice, and the results of two independent *in vivo* assays are shown in Table I. Subcutaneous inoculation of 2×10^6 KB cells induced 3 tumors out of 4 inoculations (Expt. 1) and 6 tumors out of 8 inoculations (Expt. 2) 3 months after the inoculation. By contrast, only one tumors was found out of 4 inoculations (Expt. 1) and no tumor out of 8 inoculations (Expt. 2) when 2×10^6 cells of KB-C1 were inoculated. Inoculation of 2×10^7 cells of KB-C1 induced 4 tumor out of 4 inoculations. C1-R2 cells induced 4 tumors out of 4 inoculations and 5 tumors out of 8 inoculations when a smaller number of the cells was inoculated (Table I).

DISCUSSION

Our data indicate that a classical MDR clone derived from human cancer KB cell line shows a decreased number of EGF receptors and reduced tumorigenicity in

Table I. Tumorigenicity of KB, KB-C1 and C1-R2 Transplanted in Nude Mice

Cell lines	Cell number	Incidence of tumors[a)]	
		Expt. 1	Expt. 2
KB	2×10^6	3/4	6/8
	2×10^7	—[b)]	—
KB-C1	2×10^6	1/4	0/8
	2×10^7	—	4/4
C1-R2	2×10^6	4/4	5/8
	2×10^7	—	—

a) KB, KB-C1 and C1-R2 cells either at 2×10^6 and 2×10^7 were subcutaneously inoculated into a nude mouse. The values are number of tumors appearing per number of inoculations.
b) Not tested.

comparison with the parental KB or its drug-sensitive revertant C1-R2. Biedler *et al.* have reported that the development of resistance to actinomycin D, daunomycin or vincristine in Chinese hamster cells results in lower oncogenic potential and a less malignant phenotype relative to the non-resistant counterparts.[14)] It appears that the acquisition of the MDR phenotype by tumor cells is correlated with their tumorigenicity and other tumor-related properties in certain cell lines.

Many MDR clones of Chinese hamster ovary (CHO) cell lines or human cancer cell lines exhibit increased expression of the P-glycoprotein on the cell surfaces.[3, 5, 11)] Many MDR clones, including KB-C1 cells, show decreased expression of 70,000–80,000 dalton proteins and increased expression of a 21,000 dalton protein.[11, 26)] Beck *et al.* have reported that MDR variants of a human leukemic cell line are more differentiated than the parental cell line and MDR-associated glycoproteins of 155,000 and 130,000 daltons appear during the differentiation process.[11)] Ganglioside composition is also altered in Chinese hamster MDR cell lines.[13)] During the development of the MDR phenotype, the expression of various MDR-related membranous constituents appears to be changed. Although the precise molecular mechanism for such changes is not yet understood, MDR cells appear to have a more "normal" phenotype.

In this study, we have found that KB-C1 cells show a greater resistance to the cytotoxic action of EGF and EGF-PE than KB or C1-R2 cells. Mutant KB cells with decreased EGF-receptor expression are resistant to EGF or EGF-PE,[27, 28)] suggesting a correlation between EGF receptor number and cytotoxicity by EGF-PE. A decreased expression of EGF-receptor was observed on the cell surface of KB-C1 cells (Fig. 4). Although we observed similar levels of expression of various housekeeping genes and oncogenes in the cell lines (K. Kohno, unpublished data), the mRNA level of EGF receptor gene is reduced in KB-C1 cells (Fig. 5). In contrast, Meyers *et al.* have reported increased EGF receptor in MDR clones of Chinese hamster and mouse tumor cells,[14, 15)] and a concomitant increase of EGF receptor was also observed in MDR cell lines derived from mouse and human cell lines.[29)] These MDR clones manifest a more "normalized" phenotype and a lower tumorigenic potential, and the increased expression of EGF receptor in MDR cells might be associated with reverse transformation.[29)] It remains unknown whether some drug-resistant tumor cells with high expression of EGF receptor can grow *in vivo* after prolonged chemotherapy. There appears to be no simple explanation for this discrepancy of cellular levels of EGF receptor at present. Acquisition of MDR might differentially affect the expression of the EGF receptor among the cell lines used. Ganapathi *et al.* have reported that progressive acquisition of adriamycin-resistance by mouse melanoma cells does not cause a significant change in their tumorigenicity, but dose lower pulmonary metastasis formation.[30)] The EGF receptor levels of their adriamycin-resistant melanoma cells were not measured, but the tumorigenicity of MDR clones appeared to vary among the cell lines used.

One can ask how EGF-mediated cytotoxicity occurs, and how the development of the MDR phenotype causes a decreased expression of EGF-receptor on the cell surface of KB-C1 cells. KB or C1-R2 cells show higher susceptibility to the cytotoxic action of EGF itself than KB-C1 cells. Hirai *et al.* have reported two squamous cancer cell lines with amplified EGF receptor genes whose growth is inhibited by 1–10 ng/ml EGF,[31)] and they isolated variants of EGF-dependent growth from these cell lines. They suggested that loss of amplified EGF receptor genes and efficient down-regulation of the cell surface receptor might be involved in the acquisition of EGF-resistant phenotype.[31)] We could not observe any stimulatory effect by EGF on cell growth of KB, KB-C1 and C1-R2 when 10^{-3} ng/ml to 10^{-2} ng/ml of EGF was added (see Fig. 2 and unpublished data). Further study is required to establish precisely the mechanism of EGF-induced cytotoxic effects. On the other hand, altered expression of members of a family of proteins during acquisition of MDR phenotype[10–14)] might concomitantly change EGF-receptor activity. Alternatively, development of MDR phenotype might also change the expression of EGF-receptor, resulting in altered tumorigenicity. Cytogenetic analysis has shown the EGF receptor gene to be located on human chromosome 7.[32, 33)] Acquisition of doxorubicin-resistance is associated with alterations chromosome 7, and the *mdr-1* gene is located on the same chromosome 7.[34)] Collard *et al.* have also shown that genes involved in invasion and metastasis

are located on human chromosome 7.[35] Selection of an MDR clone by exposure to colchicine after mutagenesis with ethylmethane sulfonate[16] might cause concomitant chromosomal alterations of chromosome 7 including the *mdr-1* gene and the EGF receptor gene. This possibility is still highly speculative, and further cytogenetic analysis of the MDR clones will be necessary to confirm or refute it.

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REFERENCES

1) Lane, M. Clinical problems of resistance to cancer chemotherapeutic agents. *Fed. Proc.*, **38**, 103–107 (1979).
2) Ling, V. and Baker, R. M. Dominance of colchicine resistance in hybrid CHO cells. *Somatic Cell Genet.*, **4**, 193–200 (1978).
3) Debenham, P. G., Kartner, N., Siminovitch, L., Riordan, J. R. and Ling, V. DNA mediated transfer of multidrug-resistance and plasma membrane gloycoprotein expression. *Mol. Cell Biol.*, **2**, 881–889 (1982).
4) Juliano, R. L. and Ling, V. A surface glycoprotein modulating drug permeability in Chinese hamster ovary cell mutants. *Biochim. Biophys. Acta*, **455**, 152–162 (1976).
5) Kartner, N., Riordan, J. and Ling, V. Cell surface P-glycoprotein associated with multidrug resistance in mammalian cell lines. *Sience*, **221**, 1285–1288 (1983).
6) Riordan, J. R. and Ling, V. Purification of P-glycoprotein from plasma membrane vesicles of Chinese hamster ovary cell mutants with reduced colchicine permeability. *J. Biol. Chem.*, **254**, 12701–12705 (1979).
7) Gros, P., Croop, J. and Housman, D. Mammalian multidrug-resistance gene: complete cDNA sequence indicates strong homology to bacterial transport proteins. *Cell*, **47**, 371–380 (1986).
8) Chen, C. J., Chin, E. J., Ueda, K., Clark, P. D., Pastan, I., Gottesman, M. M. and Roninson, B. I. Internal duplication and homology with bacterial transport proteins in the *mdr-1* (P-glycoprotein) gene from multidrug-resistant human cells. *Cell*, **47**, 381–389 (1986).
9) Shen, D. W., Fojo, A., Chin, J. E., Roninson, I. B., Richert, N., Pastan, I. and Gottesman, M. M. Human multidrug-resistant cell lines: increased *mdr-1* expression can precede gene amplification. *Science*, **232**, 643–645 (1986).
10) Danks, M. K., Metzger, D. W., Ashmun, R. A. and Beck, W. T. Monoclonal antibodies to glycoproteins of Vinca alkaloid-resistant human leukemic cells. *Cancer Res.*, **45**, 3220–3224 (1985).
11) Beck, W. T., Cirtain, C. M., Ashmun, A. R. and Mirro, J. Differentiation and the multiple drug resistance phenotype in human leukemic cells. *Cancer Res.*, **46**, 4571–4575 (1986).
12) Richert, N., Akiyama, S., Shen, D., Gottesman, M. M. and Pastan, I. Multiply drug-resistant human KB carcinoma cells have decreased amounts of a 75-kDa glycoprotein. *Proc. Natl. Acad. Sci. USA*, **82**, 2330–2333 (1985).
13) Peterson, R. H. F., Beutler, W. J. and Biedler, J. L. Ganglioside composition of malignant and actinomycin D-resistant nonmalignant Chinese hamster cells. *Biochem. Pharmacol.*, **28**, 579–582 (1979).
14) Biedler, L. J., Riehm, H., Peterson, H. E. R. and Spengler, A. B. Membrane-mediated drug resistance and phenotypic reversion to normal growth behavior of Chinese hamster cells. *J. Natl. Cancer Inst.*, **55**, 671–677 (1975).
15) Meyers, B. M., Merlnzzi, J. V., Spengler, A. B. and Biedler, L. J. Epidermal growth factor receptor is increased in multidrug resistant Chinese hamster and mouse tumor cells. *Proc. Natl. Acad. Sci. USA*, **83**, 5521–5525 (1986).
16) Akiyama, S., Fojo, A., Hanover, J. A., Pastan, I. and Gottesman, M. M. Isolation and genetic characterization of human KB cell line resistant to multiple drugs. *Somatic Cell Mol. Genet.*, **11**, 117–126 (1985).
17) Shiraishi, N., Akiyama, S., Nakagawa, M., Kobayashi, M. and Kuwano, M. Effect of bisbenzylisoquinoline (biscoclaurine) alkaloids on multidrug resistance in KB human cancer cells. *Cancer Res.*, **47**, 2413–2416 (1987).
18) Shiraishi, N., Akiyama, S., Hagino, Y., Kohno, K., Kobayashi, M., Kuwano, M. and Akiyama, S. Potentiation by a biscoclaurine alkaloid, cepharanthine, of the toxicity of conjugates of epidermal growth factor with *Pseudomonas* exotoxin in HeLa cells. *Cancer Res.*, **48**, 1307–1311 (1988).
19) Kuratomi, Y., Akiyama, S., Ono, M., Shiraishi, N., Shimada, T., Ohkuma, S. and Kuwano, M. Thioridazine enhances lysosomal accumulation of epidermal growth factor and toxicity of conjugates of epidermal growth factor with *Pseudomonas* exotoxin. *Exp. Cell Res.*, **162**, 436–447 (1986).
20) Das, M., Miyakawa, T., Fox, C. F., Pruso, R. M., Aharonov, A. and Herschman, H. R. Specific radiolabeling of a cell surface receptor for epidermal growth factor. *Proc. Natl. Acad. Sci. USA*, **74**, 2790–2794 (1977).

21) Maniatis, T., Fritsch, F. E. and Sambrook, J. "Molecular Cloning: A Laboratory Manual," pp. 191–193 (1982). Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
22) Merlino, G. T., Ishii, S., Whang-Peng, J., Knutsen, T., Xu, Y. H., Clark, A. J. L., Stratton, R. H., Wilson, R. K., Ma, D. P., Roe, B. A., Hunts, J. H., Shimizu, N. and Pastan, I. Structure and localization of genes encoding aberrant and normal epidermal growth factor RNAs from A431 human carcinoma cells. *Mol. Cell. Biol.*, **5**, 1722–1734 (1985).
23) Roninson, I. B., Chin, J. E., Choi, K., Gros, P., Housman, D. E., Fojo, A., Shen, D. W., Gottesman, M. M. and Pastan, I. Isolation of the human *mdr* DNA sequences amplified in multidrug-resistant KB carcinoma cells. *Proc. Natl. Acad. Sci. USA*, **83**, 4538–4542 (1986).
24) Iijima, N. S., Hamada, H., Reddy, P. and Kakunaga, T. Molecular structure of the human cytoplasmic β-actin gene: interspecies homology of sequences in the introns. *Proc. Natl. Acad. Sci. USA*, **82**, 6133–6137 (1985).
25) Feinberg, A. and Vogelstein, B. A technique for rediolabeling DNA restriction endonuclease fragments to high specific activity. *Anal. Biochem.*, **132**, 6–13 (1983).
26) Shen, D. W., Cardarelli, C., Hwang, J., Cornwell, Richert, N., Ishii, S., Pastan, I. and Gottesman, M. M. Multiple drug-resistant human KB carcinoma cells independently selected for high-level resistance to colchicine, adriamycin, or vinblastine show changes in expression of specific proteins. *J. Biol. Chem.*, **261**, 7762–7770 (1986).
27) Hwang, J., Richert, N., Pastan, I. and Gottesman, M. M. Mutant KB cells with decreased EGF receptor expression: biochemical characterization. *J. Cell. Physiol.*, **133**, 127–134 (1987).
28) Lyall, R. M., Pastan, I. and Willingham, M. C. EGF induces receptor down-regulation with no receptor recycling in KB cells. *J. Cell. Physiol.*, **122**, 166–170 (1985).
29) Biedler, J. L., Meyers, M. B. and Spengler, B. A. Cellular concomitants of multidrug resistance. *In* "Mechanisms of Drug Resistance in Neoplastic Cells," ed. P. V. Woolley III and K. D. Tew, pp. 41–68 (1988). Academic Press, Inc., New York.
30) Ganapathi, R., Grabowski, D., Schmidt, H., Bell, D. and Melia, M. Characterization *in vitro* and *in vivo* of progressively adriamycin-resistant B16-BL6 mouse melanoma cells. *Cancer Res.*, **47**, 3464–3468 (1987).
31) Hirai, M., Gamou, S., Minoshima, S. and Shimizu, N. Two independent mechanisms for escaping epidermal growth factor mediated growth inhibition in epidermal growth factor receptor-hyperproducing human tumors. *J. Cell. Biol.*, **107**, 791–799 (1988).
32) Shimizu, M., Behzadian, A. M. and Shimizu, Y. Genetics of cell surface receptors for bioactive polypeptides: binding of epidermal growth factor is associated with the presence of human chromosome 7 in human-mouse cell hybrids. *Proc. Natl. Acad. Sci. USA*, **77**, 3600–3604 (1980).
33) Koprowski, H., Herlyn, M., Balaban, G., Parmiter, A., Ross, A. and Nowell, P. Expression of the receptor for epidermal growth factor correlates with increased dosage of chromosome 7 in malignant melanoma. *Somatic Cell Mol. Genet.*, **11**, 297–301 (1985).
34) Slovak, L. M., Hoeltge, A. G. and Trent, M. J. Cytogenetic alterations associated with the acquisition of doxorubicin resistance: possible significance of chromosome 7 alterations. *Cancer Res.*, **47**, 6646–6652 (1987).
35) Collard, G. J., van de Poll, M., Schetter, A., Roos, E., Hopman, H. M. A., Geurts van Kessel H. M. A. and van Dongen, J. M. J. Location of genes involved in invasion and metastasis on human chromosome 7. *Cancer Res.*, **47**, 6666–6670 (1987).