Pergamon

Biochemical Pharmacology, Vol. 50, No. 4, pp. 475–480, 1995.

0006-2952/95 $9.50 + 0.00

0006-2952(95)00157-3

EXPRESSION OF MULTIDRUG RESISTANCE IN RESPONSE TO DIFFERENTIATION IN THE K562 HUMAN LEUKAEMIA CELL LINE

DENESE C. MARKS,* MARY W. DAVEY,*† ROSS A. DAVEY‡ and ANTONY D. KIDMAN*

*Neurobiology Unit, University of Technology, Westbourne Street, Gore Hill, Sydney, NSW 2065, Australia; and ‡Bill Walsh Cancer Research Laboratories, Royal North Shore Hospital, St. Leonards, NSW 2065, Australia

(*Received* 28 *September* 1994; *accepted* 30 *March* 1995)

Abstract—With the increasing use of inducers of cellular differentiation in the treatment of leukaemia, it is essential to understand the relationship between differentiation and the expression of the multidrug resistance. Using the K562 human leukaemia cell line and its multidrug resistant subline K562/E15B, differentiation was examined along two different pathways, megakaryocyte in response to treatment with 12-*O*-tetradecanoylphorbol-13-acetate (TPA), and erythroid in response to treatment with sodium butyrate, in the same cell line. P-glycoprotein expression was increased in the multidrug resistant K562/E15B subline, but not induced in the parental K562 cell line. However, both treatments conferred a different phenotype on the drug resistant subline. TPA treatment caused an increase in P-glycoprotein, increased drug resistance and decreased rhodamine-123 accumulation which was verapamil sensitive, demonstrating that TPA induced a fully functional P-glycoprotein. However, sodium butyrate treatment caused an increase in P-glycoprotein without increased drug resistance or without decreased rhodamine-123 accumulation suggesting that the P-glycoprotein induced by sodium butyrate was non-functional. These results stress the importance of examining not only the expression of P-glycoprotein in cells, but also the function of the P-glycoprotein induced.

Key words: multidrug resistance; P-glycoprotein; differentiation; K562; TPA; sodium butyrate

Leukaemia may be viewed as aberrant cell proliferation which can be treated with cytotoxic drugs. While this is often successful, failure of this approach is usually due to the development of MDR§ frequently characterized by the expression of a 170 kDa membrane protein P-glycoprotein [1]. This membrane protein acts as a drug efflux pump to remove cytotoxic drugs from the cell to confer resistance [2]. An alternative view of leukaemia is as aberrant differentiation in which cells fail to mature, and so continue to proliferate. This offers an alternative approach to therapy using agents that induce terminal differentiation [3]. The strategy for improving therapy could possibly lie in the combination of differentiation based therapy with conventional chemotherapy as many reports suggest that P-glycoprotein expressing sublines respond to treatment with differentiation agents the same as the parental cell, offering an alternative treatment for cytotoxic drug-resistant tumours [4, 5].

As many differentiating agents are already undergoing clinical trial [6], it is important to know if this approach to treatment may increase multidrug resistance in P-glycoprotein expressing cells. NaB and TPA are commonly used in *in vitro* studies to examine the relationship between P-glycoprotein expression and differentiation, and many reports suggest that increased differentiation with these agents is associated with increased expression of P-glycoprotein [5, 7, 8]. However, conflicting reports suggest that this increased expression is not always accompanied by increased drug resistance, and the lack of correlation between expression of P-glycoprotein and the activity of P-glycoprotein is unexplained [9, 10].

K562 is a human leukaemia cell line which offers an ideal model to study the relationship between expression of P-glycoprotein and differentiation as these cells were developed from a patient with chronic myelocytic leukaemia in blast crisis and are a pluripotent cell capable of undergoing differentiation along lineage specific pathways in response to a variety of agents [11, 12]. Previous reports demonstrate that MDR K562 cells are sensitive to induction by treatment with NaB [13], but resistant to induction by treatment with TPA [14]. As this drug resistant cell line was developed by adaptation to growth in high drug concentrations (500 ng/mL doxorubicin), the effects of induction of differentiation may not be clinically relevant. We have previously reported the development of a MDR K562 subline (K562/E15B) by treatment with low concentrations (15 ng/mL) of the anthracycline epirubicin, in an attempt to more closely mimic cellular changes associated with the clinical development of drug resistance [15]. This subline expresses

† Corresponding author. Tel. 61 2 330 4065; FAX 61 2 906 4150.

§Abbreviations: MDR, multidrug resistance; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; NaB, sodium butyrate; MTT, 3,4,5-dimethylthiazol-2,5-diphenyl tetrazolium bromide; DMSO, dimethyl sulphoxide.

P-glycoprotein and a MDR phenotype, and provides the opportunity to examine the effect of induction of differentiation along two different pathways in the same cell line. We report here on the response of these cells to the effects of NaB which induces erythroid differentiation [16] and the phorbol ester TPA which causes megakaryocytic differentiation in the parental K562 cell line [17].

MATERIALS AND METHODS

Cell culture and treatments. K562 cell line was obtained from the American Type Culture Collection, and the K562/E15B subline was derived from K562 cells in our laboratory by intermittent treatment with 15 ng/mL epirubicin [16]. The cells were grown in RPMI-1640 supplemented with 10% foetal bovine serum, 20 mM HEPES and $NaHCO_3$ (0.85 g/L) at 37° in a humidified atmosphere with 5% CO_2. All cultures were free of mycoplasma.

TPA (Sigma, St Louis, MO, U.S.A.) was stored at −20° as a stock solution in DMSO, and diluted in RPMI before use. Treatments were for 18 hr with 5 nM TPA. Control treatments used the carrier alone. NaB (Sigma) was made fresh for each experiment and was dissolved in RPMI. Cells were treated with 1.5 and 15 mM NaB for 18 hr. All treatments used exponentially growing cells at 2×10^5 cells/mL.

Cytotoxicity assays. After treatment with the differentiating agent, cells (3×10^4 cells/well) were plated in triplicate into 96-well microtitre plates (Costar, MA, U.S.A.) in 200 μL growth medium containing serial two-fold dilutions of the drugs epirubicin (Farmitalia, Italy), vinblastine (David Bull, Vic, Australia) and colchicine (Sigma). Cells were incubated for 4 days after which cell viability was determined using the MTT cell viability assay [18].

Detection of P-glycoprotein. C219 monoclonal antibody (Centocore, Malvern, PA, U.S.A.) was used to detect P-glycoprotein according to the method previously described [19]. Protein (2 μg) was loaded for each sample and equal protein loading was confirmed by Coomassie staining of duplicate gels.

Flow cytometry. Cells (10^5) were incubated with antibodies to CD61 (3.15 μg/mL), glycophorin A (2.66 μg/mL; both from Dako, Glostrup, Denmark) or control mouse IgG1 antibody (raised against a κ myeloma antibody, kindly donated by Prof. R. Raison, Immunobiology Unit, University of Technology, Sydney, Australia) as previously described [15].

Rhodamine-123 accumulation. P-glycoprotein function was measured by the ability of cells to accumulate rhodamine-123 (Sigma) in the presence or absence of 10 μM verapamil (Sigma). Rhodamine-123 was dissolved in ethanol and stored as a stock solution (5 mg/mL) at 4°. Cells (5×10^5) were incubated at 37° for 45 min with rhodamine-123 at a final concentration of 150 ng/mL. For treatment with verapamil, 10 μM verapamil was added 10 min prior to the addition of rhodamine-123. Cells were then washed once with ice-cold PBS, resuspended in ice-cold PBS containing 50 μg/mL propidium iodide (Sigma) and fluorescence analysed immediately using a Becton Dickinson FACScan flow cytometer as previously described [20]. Dead cells which took up propidium iodide were excluded from the analysis by gating. All determinations were in duplicate and experiments were repeated at least three times.

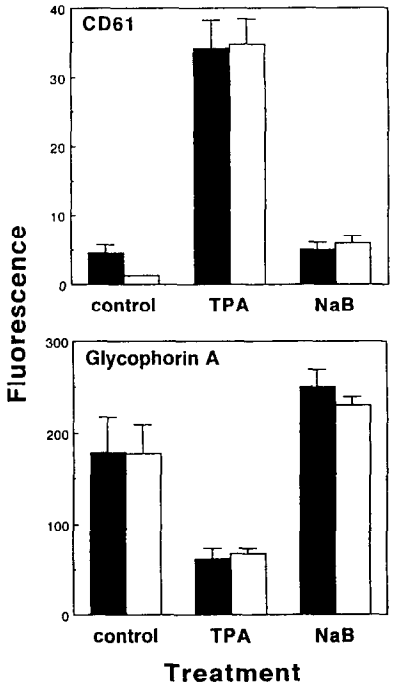

Fig. 1. Flow cytometry analysis of CD61 and glycophorin A after TPA and NaB treatment. K562 cells (solid bar) and the K562/E15B subline (open bar) were analysed for expression of CD61 and glycophorin A after treatment for 18 hr with 5 nM TPA or after 4 day treatment with 1.5 mM NaB. Results are the mean of three experiments; error bars are the standard error of the mean.

RESULTS

Differentiation

Treatment of both K562 and K562/E15B cells with TPA (5 nM) for 18 hr increased the expression of platelet glycoprotein IIIa (CD61) normally expressed by megakaryocytes and platelets, and decreased the expression of glycophorin A, expressed by cells of the erythroid lineage (Fig. 1). Similar results were found at 1 and 16 nM TPA (results not shown). While treatment with NaB (1.5 mM) resulted in little change in CD61 or glycophorin A expression after 18 hr (results not shown), increased expression of glycophorin A was demonstrated after 4 days treatment of both K562 and K562/E15B cells, suggesting differentiation along the erythroid pathway (Fig. 1).

P-glycoprotein expression

Treatment of the K562/E15B subline with either TPA or NaB for 18 hr increased P-glycoprotein

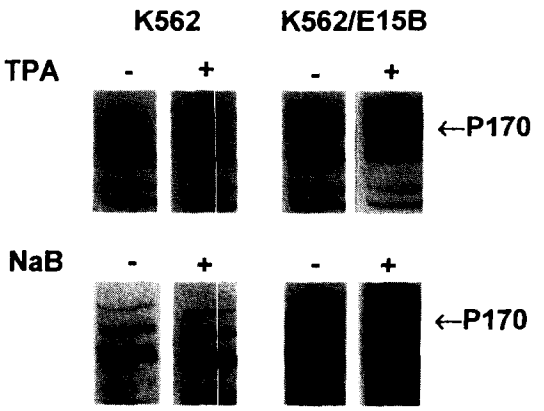

Fig. 2. Western blot analysis of P-glycoprotein. Cells were treated for 18 hr with 5 nM TPA or 1.5 mM NaB after which plasma membranes were prepared and analysed using C219 monoclonal antibody. Position of P-glycoprotein (P-170) is indicated.

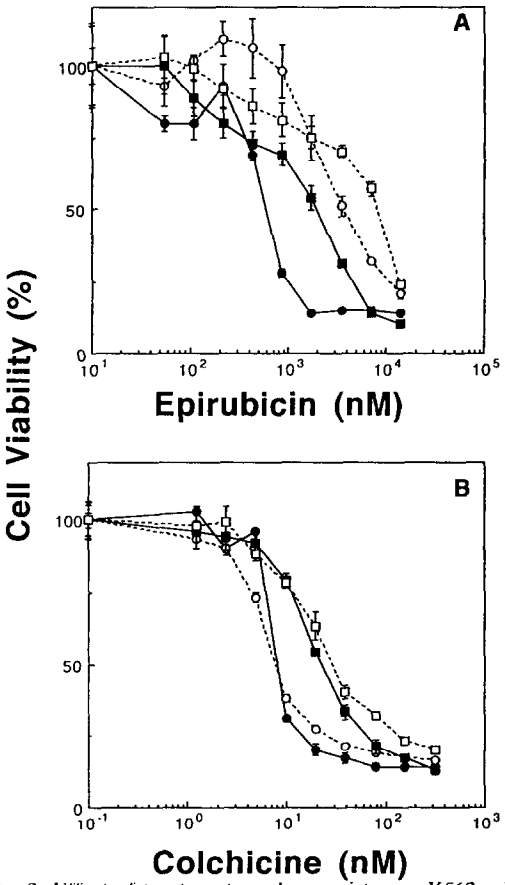

Fig. 3. Effect of treatment on drug resistance. K562 cells (○, ●) and the K562/E15B subline (□, ■) were treated with (A) 5 nM TPA or (B) 1.5 mM NaB for 18 hr (----) and the resistance compared to untreated cells (——). Points are the mean of triplicate determinations and error bars show standard deviation. Experiments were repeated at least three times and representative results are shown.

expression as determined by Western blot analysis using the monoclonal antibody C219 (Fig. 2). There was no induction of P-glycoprotein in the K562 cells with either TPA or NaB.

Drug resistance

As P-glycoprotein expression was increased by TPA and NaB treatment after 18 hr, drug resistance was examined at this time. Treatment with 5 nM TPA caused a 4.9 ± 2.7-fold increase in resistance to epirubicin (Fig. 3A) and a 27.5 ± 23-fold increase in resistance to vinblastine in the K562 cells (results not shown), while for the K562/E15B subline it caused a 2.8 ± 1.6-fold increase in resistance to epirubicin (Fig. 3A) and a 3.1 ± 1.4-fold increase in resistance to vinblastine (results not shown; N = 3). TPA at this concentration did not inhibit cell proliferation.

Although 1.5 mM NaB treatment caused increased P-glycoprotein expression in the drug resistant subline, there was little if any effect on the sensitivity to epirubicin (results not shown) or colchicine (Fig. 3B) in either the K562 cells or the K562/E15B subline. Treatment with 15 mM NaB, which gave a similar increase in P-glycoprotein expression by Western blot analysis, gave similar results with little effect on drug resistance (<2-fold change; results not shown).

Function of P-glycoprotein

Since increased P-glycoprotein expression did not always result in increased resistance, P-glycoprotein function was assessed using rhodamine-123 accumulation which is a measure of P-glycoprotein mediated drug efflux [21]. The P-glycoprotein expressing multidrug resistant K562/E15B subline showed a significant decrease in rhodamine-123 accumulation compared to the parental K562 cells (49.2 ± 8.6% of K562 accumulation; $P < 0.001$; Fig. 4). TPA treatment decreased the rhodamine-123 accumulation in both the K562 cells (63.5 ± 9.8%; $P < 0.01$), and further significantly decreased the rhodamine-123 accumulation in the K562/E15B subline to 17.2 ± 2.6% of the accumulation in the untreated K562 cells ($P < 0.01$). Thus the increased P-glycoprotein expression in the drug resistant subline demonstrated by the Western blot was functional and able to reduce rhodamine accumulation.

The function of P-glycoprotein induced by TPA was further examined by treatment of cells with verapamil, an inhibitor of P-glycoprotein mediated drug efflux. In the presence of verapamil, the K562/E15B subline showed increased accumulation of rhodamine-123, a characteristic of P-glycoprotein mediated drug efflux ($P < 0.001$; Fig. 4). However, verapamil had no effect on rhodamine-123 accumulation in the K562 cells after treatment with TPA, but did increase the accumulation in the K562/E15B subline, indicating that the P-glycoprotein induced by TPA was functional (Fig. 4).

Treatment of K562 cells or the drug resistant subline with 1.5 mM NaB did not cause any change in rhodamine-123 accumulation (Fig. 4) which is

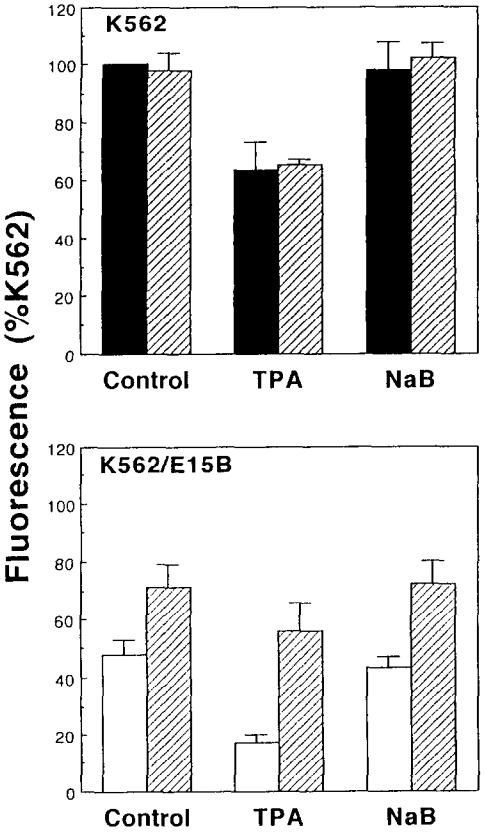

Fig. 4. Effect of TPA and NaB on rhodamine-123 accumulation. K562 cells (solid bar) and the K562/E15B subline (open bar) were treated with 5 nM TPA or 1.5 mM NaB for 18 hr and rhodamine-123 accumulation measured by flow cytometry in the absence or presence (hatched bars) of 10 μM verapamil. Results are the mean of $\geq$ three experiments, calculated as a percentage of the untreated K562 cells; error bars are the standard error of the mean.

consistent with its lack of effect on drug resistance. Similar results were obtained using treatment with 15 mM NaB (results not shown).

DISCUSSION

Treatment of the K562 parental cell line and the multidrug resistant subline K562/E15B with TPA produced a megakaryocytic phenotype with increased expression of the platelet glycoprotein IIIa (CD61) and decreased expression of the erythroid antigen glycophorin A as reported by Yen and co-workers [17]. The presence of a MDR phenotype had no effect on TPA induced differentiation as the P-glycoprotein-expressing K562/E15B subline responded in the same way as the parental K562 cells to TPA treatment (Fig. 1). Induction of erythroid differentiation (increased glycophorin A expression; Fig. 1) by NaB in both the K562 cells and the MDR subline also shows that the low level MDR K562/E15B subline retains its ability to differentiate in response to these inducers in the same manner as the parental cell line which is similar to that reported for some other MDR K562 cell lines [4, 13, 22, 23].

Differentiation induced by both TPA and NaB was accompanied by increased expression of P-glycoprotein only in the P-glycoprotein expressing multidrug resistant subline K562/E15B but not in the parental K562 cells (Fig. 2). This suggests that increased P-glycoprotein expression may be associated with a more differentiated phenotype only in cells already expressing P-glycoprotein. Although the K562 cells underwent differentiation and became more resistant to epirubicin (Fig. 3) and vinblastine in response to TPA treatment, this was not accompanied by increased P-glycoprotein expression (Fig. 2). Further, the decrease in rhodamine-123 accumulation was not verapamil sensitive (Fig. 4) consistent with this multidrug resistance not being mediated by P-glycoprotein. While TPA treatment of K562 cells has previously been reported to induce P-glycoprotein expression and function [8], high concentrations of TPA were used (30 nM) which would be toxic to the cells. Induction of P-glycoprotein under these conditions may be due to a stress response by the cells as demonstrated for other human cells lines [24].

An alternative mechanism to explain the increased drug resistance in the K562 cell line is that TPA also activates protein kinase C [25] and treatment with this and other phorbol esters has been reported to increase resistance to anticancer drugs in human breast cancer MCF-7 cells [26] and human KB cells [27] without increased P-glycoprotein expression. Transfection of the PKC α has been shown to increase multidrug resistance [28], and a similar activation of PKC may cause the increase in drug resistance in the TPA treated K562 cell line.

Although both TPA and NaB treatment increased P-glycoprotein expression in the MDR K562/E15B subline, the resulting MDR phenotype was different with both functional and non-functional P-glycoprotein being induced in the same MDR cell line. The P-glycoprotein induced by TPA was fully functional as determined by resistance to epirubicin (Fig. 3) and vinblastine and by verapamil sensitive reduction in rhodamine-123 accumulation (Fig. 4). However the P-glycoprotein induced by NaB was not functional. It did not confer increased drug resistance to epirubicin or specifically to colchicine, as described by Bates and co-workers [10]. After treatment with 1.5 mM NaB the K562/E15B subline exhibited only a slight decrease in rhodamine-123 accumulation (Fig. 4). Similarly in colon [5] and neuroblastoma cell lines [9] treatment with NaB resulted in the induction of non-functional P-glycoprotein. This is consistent with NaB increasing the expression of P-glycoprotein but also inhibiting the post-translational phosphorylation required for activity, since NaB has been shown to inhibit phosphorylation of P-glycoprotein and this may render it inactive [10]. TPA on the other hand, activates protein kinase C which has been shown to phosphorylate P-glycoprotein [29]. Thus the P-glycoprotein induced in response to TPA treatment is able to undergo post-translational phosphorylation and become fully active.

An alternative explanation for the inactivity of

the P-glycoprotein seen in the Western blot analysis (Fig. 2) is that the P-glycoprotein induced by NaB may be the product of the *mdr2* gene (also known as the *mdr3* gene) as this is also recognized by the monoclonal antibody C219, and the expression of the *mdr2* gene does not confer drug resistance [30]. This induction of non-functional P-glycoprotein may explain the poor clinical correlation often reported between expression of P-glycoprotein and response to treatment [31], and the lack of verapamil sensitivity to drug uptake found even in the presence of P-glycoprotein expressing cells [32].

The MDR phenotype, expressed by the K562/E15B subline in response to intermittent treatment with 15 ng/mL epirubicin, did not cause any cross-resistance to the inducing activity of TPA or NaB. Thus, the induction of terminal differentiation could offer an alternative treatment for P-glycoprotein expressing leukaemia, since expression of P-glycoprotein has been shown to decrease response to cytotoxic drugs [33]. However, understanding the response of drug-resistant cells to different inducers is important as we have previously demonstrated that MDR HL60 cells are not responsive to induction with retinoic acid [20], a treatment that has proved highly successful for acute promyelocytic leukaemia [3]. These studies demonstrate that the response to induction of differentiation can be an increase in expression of P-glycoprotein, although not necessarily an increase in the MDR phenotype, which may explain some of the conflicting reports in the literature on the relationship between P-glycoprotein expression and cellular differentiation. This report therefore stresses the importance when determining the effects of clinically relevant differentiating agents on leukaemia cells to examine not only the expression of P-glycoprotein, but the phenotype of the resulting cells. Further, *in vitro* testing using rhodamine-123 accumulation in combination with verapamil may be more successful in the diagnosis of drug resistance in leukaemia patients than determination of the expression of P-glycoprotein.

REFERENCES

1. Ma DDF, Davey RA, Harman DH, Isbister JP, Scurr RD, Mackertich SM, Dowden G and Bell DR, Detection of a multidrug resistant phenotype in acute non-lymphoblastic leukaemia. *Lancet* **i**: 135–137, 1987.
2. Gottesman MM and Pastan I, Biochemistry of multidrug resistance mediated by the multidrug transporter. *Ann Rev Biochem* **62**: 385–427, 1993.
3. Degos L, Differentiating agents in the treatment of leukemia. *Leuk Res* **14**: 717–719, 1990.
4. Okabe-Kado J, Hayashi M, Honma Y, Hozumi M and Tsuruo T, Effects of inducers of erythroid differentiation of human leukemia K562 cells on vincristine-resistant K562/VCR cells. *Leuk Res* **7**: 481–485, 1983.
5. Mickley LA, Bates SE, Richert ND, Currier S, Tanaka S, Foss F, Rosen N and Fojo AT, Modulation of the expression of a multidrug resistance gene (mdr-1/P-glycoprotein) by differentiating agents. *J Biol Chem* **264**: 18031–18040, 1989.
6. Smith MA, Parkinson DR, Cheson BD and Friedman MA, Retinoids in cancer therapy. *J Clin Oncol* **10**: 839–864, 1992.
7. O'Brian CA, Fan D, Ward NE, Dong Z, Iwamoto L, Gupta KP, Earnest LE and Fidler IJ, Transient enhancement of multidrug resistance by the bile acid deoxycolate in murine fibrosarcoma cells *in vitro*. *Biochem Pharmacol* **41**: 797–806, 1991.
8. Chaudhary PM and Roninson IB, Activation of MDR1 (P-glycoprotein) gene expression in human cells by protein kinase C agonists. *Oncol Res* **4**: 281–290, 1992.
9. Bates SE, Mickley LA, Chen Y-N, Richert N, Rudick J, Biedler JL and Fojo AT, Expression of drug resistance gene in human neuroblastoma cell lines: modulation by retinoic acid-induced differentiation. *Molec Cell Biol* **9**: 4337–4344, 1989.
10. Bates SE, Currier SJ, Alvarez M and Fojo AT, Modulation of P-glycoprotein phosphorylation and drug transport by sodium butyrate. *Biochemistry* **31**: 6366–6372, 1992.
11. Sutherland JA, Turner AR, Mannoni P, McGann LE and Turc J-M, Differentiation of K562 leukemia cells along erythroid, macrophage, and megakaryocyte lineages. *J Biol Response Modifiers* **5**: 250–262, 1986.
12. Leary JF, Ohlsson-Wilhelm BM, Giuliano R, LaBella S, Farley B and Rowley PT, Multipotent human haematopoietic cell line K562: Lineage-specific constitutive and inducible antigens. *Leuk Res* **11**: 807–815, 1987.
13. Shibata H, Kanamaru R, Sato T, Ishioka C, Konishi Y, Ishikawa A, Wakui A and Tsuruo T, Increase in the level of P-glycoprotein mRNA expression in multidrug-resistant K562 cell lines treated with sodium butyrate is not accompanied with erythroid differentiation. *Jpn J Cancer Res* **81**: 1214–1217, 1990.
14. Nishio K, Sugimoto Y, Nakagawa K, Niimi S, Fujiwara Y, Bungo M and Kasahara K, Cross-resistance to tumour promotors in human cancer cell lines resistant to adriamycin or cisplatin. *Br J Cancer* **62**: 415–419, 1990.
15. Marks DC, Davey MW, Davey RA and Kidman AD, Differentiation and multidrug resistance in response to drug treatment in the K562 human leukaemia cell line. *Br J Haematol* **84**: 83–89, 1993.
16. Rowley PT, Ohlsson-Wilhelm BM, Farley BA and LaBella S, Inducers of erythroid differentiation in K562 human leukemia cells. *Exper Haematol* **9**: 32–37, 1981.
17. Yen A, Varvayanis S and Platko JD, 12-*O*-Tetradecanoylphorbol-13-acetate and staurosporine induce increased retinoblastoma tumor suppressor gene expression with megakaryocytic differentiation of leukemic cells. *Cancer Res* **53**: 3085–3091, 1993.
18. Marks DC, Belov L, Davey MW, Davey RA and Kidman AD, The MTT cell viability assay for cytotoxicity testing in multidrug resistant human leukemic cells. *Leuk Res* **16**: 1165–1173, 1992.
19. Haber M, Norris MD, Kavallaris M, Bell DR, Davey RA, White L and Stewart BW, Atypical multidrug resistance in a therapy-induced, drug resistant, human leukaemic cell line (LALW-2): Resistance to *Vinca* alkaloids independent of P-glycoprotein. *Cancer Res* **49**: 5281–5287, 1989.
20. Su GMI, Davey MW, Davey RA and Kidman AD, Development of extended multidrug resistance in HL60 promyelocytic leukemia cells. *Br J Haematol* **88**: 566–574, 1994.
21. Ludescher C, Gattringer C, Drach J, Hofmann J and Grunicke H, Rapid functional assay for the detection of multidrug-resistant cells using the fluorescent dye rhodamine 123. *Blood* **78**: 1385–1387, 1991.
22. Hait WN, Choudhury S, Srimatkandada S and Murren JR, Sensitivity of K562 human chronic myelogenous leukemia blast cells transfected with a human multidrug resistance cDNA to cytotoxic drugs and differentiation. *J Clin Invest* **91**: 2207–2215, 1993.
23. Okabe-Kado J, Hayashi M, Honma Y, Hozumi M and Tsuruo T, Inhibition by erythroid differentiation factor

(Activin A) of P-glycoprotein expression in multidrug-resistant human K562 erythroleukemia cells. *Cancer Res* **51**: 2582–2586, 1991.

24. Chin K-V, Chauhan SS, Pastan I and Gottesman MM, Regulation of mdr RNA levels in response to cytotoxic drugs in rodent cells. *Cell Growth Diff* **1**: 361–365, 1990.
25. Kikkawa U, Kishimoto A and Nishizuka Y, The protein kinase C family: Heterogeneity and its implications. *Ann Rev Biochem* **58**: 31–44, 1989.
26. Fine RL, Patel J and Chabner BA, Phorbol esters induce multidrug resistance in human breast cancer cells. *Proc Natl Acad Sci USA* **85**: 582–586, 1988.
27. Ferguson PJ and Cheng Y-C, Transient protection of cultured human cells against antitumor agents by 12-*O*-tetradecanoylphorbol-13-acetate. *Cancer Res* **47**: 433–441, 1987.
28. Yu G, Ahmad S, Aquino A, Fairchild CR, Trepel JB, Ohno S, Suzuki K, Tsuruo T, Cowan KH and Glazer RI, Transfection with protein kinase C-alpha confers increased multidrug resistance to MCF-7 cells expressing P-glycoprotein. *Cancer Commun* **3**: 181–189, 1991.
29. Chambers TC, Pohl J, Raynor RL and Kuo JF, Identification of specific sites in human P-glycoprotein phosphorylated by protein kinase C. *J Biol Chem* **268**: 4592–4595, 1993.
30. Schinkel AH, Roelofs ME and Borst P, Characterization of the human MDR3 P-glycoprotein and its recognition by P-glycoprotein-specific monoclonal antibodies. *Cancer Res* **51**: 2628–2635, 1991
31. Arceci RJ, Clinical significance of P-glycoprotein in multidrug resistance malignancies. *Blood* **81**: 2215–2222, 1993.
32. Marie J-P, Helou C, Thevenin D, Delmer A and Zittoun R, *In vitro* effect of P-glycoprotein (P-Gp) modulators on drug sensitivity of leukemic progenitors (CFU-L) in acute myelogenous leukemia (AML). *Exper Haematol* **20**: 565–568, 1992.
33. Sato H, Gottesman MM, Goldstein LJ, Pastan I, Block AM, Sandberg AA and Preisler HD, Expression of the multidrug resistance gene in myeloid leukemias. *Leuk Res* **14**: 11–22, 1990.