Human P-glycoprotein Transports Cortisol, Aldosterone, and Dexamethasone, but Not Progesterone*

Kazumitsu Ueda, Noboru Okamura, Midori Hira, Yusuke Tanigawara, Tohru Saeki, Noriyuki Kioka, Tohru Komano, and Ryohei Horii

From the Laboratory of Biochemistry, Department of Agricultural Chemistry, Faculty of Agriculture and the Department of Pharmacy, Kyoto University Hospital, Faculty of Medicine, Kyoto University, Kyoto 606-01, Japan

We expressed human MDR1 cDNA isolated from the human adrenal gland in porcine LLC-PK1 cells. A highly polarized epithelium formed by LLC-GA5-COL300 cells that expressed human P-glycoprotein specifically on the apical surface showed a multidrug-resistant phenotype and had 8.3-, 3.4-, and 6.5-fold higher net basal to apical transport of 3H-labeled cortisol, aldosterone, and dexamethasone, respectively, compared with host cells. But progesterone was not transported, although it inhibited azidopine photoaffinity labeling of human P-glycoprotein and increased the sensitivity of multidrug-resistant cells to vinblastine. An excess of progesterone inhibited the transepithelial transport of cortisol by P-glycoprotein. These results suggest that cortisol and aldosterone are physiological substrates for P-glycoprotein in the human adrenal cortex and that substances that efficiently bind to P-glycoprotein are not necessarily transported by P-glycoprotein.

P-glycoprotein acts as an energy-dependent efflux pump that exports anticancer agents out of the cell, lowering their intracellular concentration to sublethal levels, and is considered to be important in multidrug resistance of human tumors (1, 2). P-glycoprotein is expressed in normal human tissues and is found on the luminal surface of transporting epithelia of the kidney proximal tubule, small intestine, colon, and liver biliary hepatocytes and in capillary endothelial cells of the brain and testis as well as in the adrenal cortex (3–5). The location of P-glycoprotein expression suggests that one of the physiological roles of P-glycoprotein is the secretion of metabolites and natural toxic substances into bile and urine and directly into the lumen of the gastrointestinal tract. It is important to identify the physiological substrates to predict the side effects that may arise from preventing the function of P-glycoprotein in chemotherapy, but no physiological substrates for P-glycoprotein to transport have been identified.

P-glycoprotein is extensively expressed in the human adrenal cortex (3, 4). In the pregnant mouse, mdrlb gene product is found at high levels in the gravid uterus (6, 7). These results suggest that steroids could be physiological substrates for P-glycoprotein. In particular, progesterone is a candidate because it is very active in 1) inhibiting [3H]azidopine photoaffinity labeling of P-glycoprotein in multidrug-resistant (MDR)1 cells (6–8), 2) inhibiting [3H]vinblastine or vincristine binding to the plasma membrane of MDR cells (7–9), 3) enhancing the reduced uptake of vinblastine in MDR cells (8), and 4) increasing the sensitivity of MDR cells to vinblastine (7, 8) and because 5) progesterone itself photoaffinity labels P-glycoprotein (10). But it is not clear whether P-glycoprotein transports steroids because verapamil-sensitive transport of progesterone could not be measured in NIH-3T3 transfomants expressing human P-glycoprotein, and the steady-state level of progesterone accumulation has been suggested to be very similar in drug-sensitive and -resistant rodent cells (8), and apparently no efflux of progesterone is observed in rodent MDR cells (7).

In this study, we expressed human MDR1 cDNA isolated from the human adrenal gland in porcine LLC-PK1 cells and found that P-glycoprotein transepithelially transports cortisol, aldosterone, and dexamethasone, but not progesterone. Our results suggest that these steroid hormones could be physiological substrates for P-glycoprotein in the human adrenal cortex.

**EXPERIMENTAL PROCEDURES**

**Materials**—Monoclonal antibody C219 was purchased from Centocor. Monoclonal antibody MRK16 was a generous gift from Dr. Takashi Tsuruo, Tokyo University, Japan. Deoxycoformycin and 9-β-d-xylofuranosyladenine were obtained from the National Cancer Institute. [3H]Azidopine (1850 GBq/mmol) was purchased from Amersham Corp., [3H]Cortisol (340 GBq/mmol), [3H]Cortisol (3256 GBq/mmol), [3H]Aldosterone (3104 GBq/mmol), and [3H]Dexamethasone (1480 GBq/mmol), and [125I]Clelinulin (0.38 GBq/g) were purchased from Du Pont-New England Nuclear.

**Cell Culture and Transfection**—LLC-PK1 cells were obtained from American Type Culture Collection and propagated in M199 medium supplemented with 10% fetal calf serum. A human P-glycoprotein expression vector (pSKGA) was constructed by replacing the MDR1 cDNA isolated from human MDR cell line KBX2 (11) in plasmid pSK1.MDR (12) with the MDR1 cDNA isolated from a normal adrenal gland (13). LLC-PK1 cells were transfected with pSKGA by the calcium phosphate coprecipitation method (14). Cells were first selected in 10 nM 2′-deoxycoformycin and 4 μM 9-β-d-xylofuranosyladenine (15) for 2 weeks. One transformant (LLC-GA5) was further selected in 10 nM 2′-deoxycoformycin and 4 μM 9-β-d-xylofuranosyladenine (15) for 2 weeks. One transformant (LLC-GA5) was further selected in stepwise increasing concentrations (20, 40, 80, 150, and 300 ng/ml of colchicine). LLC-GA5-COL300 cells were selected at 300 ng/ml colchicine and maintained at 300 ng/ml colchicine.

**Immunoblotting and [3H]Azidopine Photoaffinity Labeling**—Cells were broken in isotonic buffer by nitrogen cavitation, and membrane vesicles were obtained by sucrose gradient centrifugation (16). Membrane vesicles (50 μg of protein) prepared from LLC-PK1 and LLC-GA5-COL300 were resolved by SDS-polyacrylamide gel electrophoresis on 7% gels, electroblotted onto a nitrocellulose filter, and probed with anti-P-glycoprotein monoclonal antibody C219. Detection was

* This work was supported by grants from the Ministry of Education, Science, and Culture of Japan. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 The abbreviation used is: MDR, multidrug-resistant.

2 K. Ueda, N. Okamura, M. Hira, Y. Tanigawara, T. Saeki, N. Kioka, T. Komano, and R. Horii, unpublished data.
done with the enhanced chemiluminescence method from Amersham Corp. Photoaffinity labeling was done as described previously (17) with minor modifications. Briefly, membrane vesicles corresponding to 10 μg of protein were reacted with 0.2 μM [3H]azidopine in the presence or absence of a 1000-fold excess (200 μM) of steroids or vinblastine at room temperature for 20 min. The reaction mixture was then irradiated with a UV lamp (Ultra-Violet Products, Blak Ray Type XX-15L) for 30 min on ice. Labeled proteins were separated by SDS-polyacrylamide gel electrophoresis on 7% gels.

Transmembrane Transport of [3H]-Labeled Vinblastine and Steroids —Cells were seeded on microporous polycarbonate membrane filters (3.0-μm pore size, 24.5-mm diameter, Transwell 3414, Coster) at a cell density of 4 × 10^4 and 5 × 10^4 cells/cm² for LLC-PK1 and LLC-GA5-COL300, respectively. The cells were grown for 3 days; and 6 h before transport experiments, the culture medium was replaced with fresh medium without colchicine. For measurement of transepithelial transport, the medium in either the basal or apical side of the monolayers was replaced with 2 ml of medium containing 3H-labeled vinblastine or steroid and 0.66 μM [14C]labeled inulin. The cells were incubated at 37 °C; an aliquot (25 μl) of the medium in the other side was taken at 1, 2, and 3 h, and the appearance of radioactivities in the other side was measured and presented as the fraction percent of the total radioactivity. Each directional transport was measured with more than three filter-bottomed cups and presented with standard error. The paracellular fluxes monitored by the appearance of [14C]inulin in the other side were 1.5% of the total radioactivity per h.

Effect of Progesterone on Drug Resistance —The IC₅₀ (the drug concentration that inhibits cell growth by 50% after 72 h) for LLC-GA5-COL300 cells was determined by a colorimetric assay using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (18) from dose-response curves using increasing concentrations of colchicine and because no amplification of porcine mdr gene(s) was observed in LLC-GA5-COL300 by Southern hybridization with the human MDR1 probe, which could detect porcine mdr gene(s) by cross-hybridization (data not shown), the P-glycoprotein expressed in LLC-GA5-COL300 cells reacted with C219 is considered to be mainly the human one. Immunostaining using monoclonal antibody MRK16, which reacts selectively with human P-glycoprotein, confirmed the high expression of human P-glycoprotein in the plasma membrane of LLC-GA5-COL300 (data not shown), and electron microscopic immunocytochemistry using MRK16 showed that human P-glycoprotein is specifically expressed on the apical surface of the cells (Fig. 2), as previously reported using Madin-Darby canine kidney cells as host cells (24).

Transmembrane Transport of Vinblastine in Monolayers of LLC-PK1 and LLC-GA5-COL300 —The substrates for P-glycoprotein are highly lipophilic and so enter cells freely through both surfaces. If there is no specific transport system, basal to apical and apical to basal transport will be the same. However, because human P-glycoprotein localizes specifically in the apical plasma membrane in LLC-GA5-COL300 cells, substrates for P-glycoprotein entering the cell from the basal surface will be expelled from the apical surface, and those entering the apical plasma membrane are likely to be caught and pumped back to the medium by P-glycoprotein, resulting in a net basal to apical flow of substrates as shown in the model (Fig. 3A). In LLC-GA5-COL300 cells, basal to apical transport of vinblastine increased 2-fold, and apical to basal transport decreased 1.5–2-fold compared to transport in LLC-PK1 cells, resulting in a 6–10-fold higher net basal to apical transport (Fig. 3B).

Transmembrane Transport of Steroid Hormones —By using this transepithelial transport system in epithelia formed by LLC-PK1 and LLC-GA5-COL300, we measured transport of 3H-labeled cortisol, aldosterone, progesterone, and dexamethasone (Fig. 4). In LLC-GA5-COL300 cells, basal to apical transport of cortisol, aldosterone, and dexamethasone increased and apical to basal transport decreased compared to transport in LLC-PK1 cells, resulting in 8.3-, 3.4-, and 6.5-fold higher net basal to apical transport of cortisol, aldosterone, and dexamethasone, respectively, than host cells. Transepithelial transport of cortisol, aldosterone, and dexamethasone was inhibited by verapamil (Fig. 5), a competitive inhibitor of P-glycoprotein (25), which confirmed that steroid

![Fig. 1. A, immunoblotting of membrane fractions from LLC-PK1 (lane 1) and LLC-GA5-COL300 (lane 2). Twenty μg of membrane protein was added to each lane and reacted with monoclonal antibody C219 as a probe for P-glycoprotein. B, azidopine photo-labeling of P-glycoprotein. Ten μg of membrane protein from LLC-PK1 (lane 1) and LLC-GA5-COL300 (lanes 2–8) was photolabeled with [3H]azidopine in the absence of competitors (lane 2) or in the presence of 200 μM (1000-fold excess) aldosterone (lane 3), cortisol (lane 4), dexamethasone (lane 5), progesterone (lane 6), testosterone (lane 7), and vinblastine (lane 8). Molecular size standards are indicated in kilodaltons to the left.](image)
Human P-glycoprotein as a Steroid Transporter

FIG. 2. Cytochemical localization using electron microscopy in LLC-GA5-COL300 cells. Sections (200 nm thick) were analyzed, and parts of the apical surface were enlarged. While on the average 30–40 gold grains were found in the apical surface of a cell, no grains were found in the basal surface.

FIG. 3. A, scheme illustrating the transcellular transport of substrates for human P-glycoprotein. B, transepithelial transport of [3H]vinblastine (12.5 nM) in wild-type LLC-PK1 (○, △) and LLC-GA5-COL300 (●, ▲) monolayers. ○ and ●, basal to apical transport; △ and ▲, apical to basal transport. Each directional transport was measured with more than three filter-bottomed cups.

transport is mediated by human P-glycoprotein. But transepithelial transport of progesterone in LLC-GA5-COL300 was not higher than that in host cells. We examined transepithelial transport of progesterone ranging in concentrations from 1.2 nM (one-tenth the concentration of that used in Fig. 4) to 50 μM, a concentration which increased the sensitivity of LLC-GA5-COL300 cells to vinblastine (Fig. 6A) and inhibited the transepithelial transport of cortisol (Fig. 7). But transepithelial transport of progesterone in LLC-GA5-COL300 and host cells was indistinguishable from that shown in Fig. 4; thus, progesterone in a range of physiological concentrations cannot be transported by P-glycoprotein. This is consistent with the lack of apparent efflux of progesterone from rodent MDR cells reported by Yang et al. (7). These results suggest that human P-glycoprotein transports cortisol, aldosterone, and dexamethasone, but does not transport progesterone.

Interaction of Steroid Hormones with Human P-glycoprotein—Progesterone was considered to be a physiological substrate because it binds to P-glycoprotein (8, 10), inhibits vincristine binding to P-glycoprotein (9), and inhibits transport of vinblastine by P-glycoprotein in MDR cells (7, 8). However, P-glycoprotein expressed in the apical membranes of LLC-GA5-COL300 cells did not transport progesterone. The inhibition of [3H]azidopine photoaffinity labeling and the increase of sensitivity to vinblastine by progesterone were shown with mouse mdr1b-encoded P-glycoprotein and mouse MDR cells (7, 8). The inhibition of the ATP-dependent binding of vincristine was examined with the plasma membrane of adriamycin-resistant variants of human myelogenous leukemia K562 cells (9). Mouse mdr1b-encoded P-glycoprotein and human MDR1-encoded P-glycoprotein expressed in the plasma membrane of human myelogenous leukemia cells and those in the apical membrane of porcine kidney cells could behave differently when interacting with progesterone. We therefore examined the effects of steroids on azidopine photoaffinity labeling and sensitivity to vinblastine using LLC-GA5-COL300 cells.

Progesterone strongly inhibited azidopine photoaffinity labeling of human P-glycoprotein in the apical membrane of porcine cells, and aldosterone and testosterone weakly inhibited it, whereas cortisol and dexamethasone, even at 1000-fold, scarcely inhibited it (Fig. 1B). These results were consistent with the apparent inhibitory constants of steroid hormones on the ATP-dependent binding of vincristine reported by Naito et al. (9). The apparent inhibitory constants of progesterone, testosterone, and cortisol were reported to be 7, 20, and >200 μM, respectively. These results indicate that progesterone has a high affinity for human P-glycoprotein whereas cortisol and dexamethasone have very low affinities for human P-glycoprotein.

The ability of progesterone or cortisol to reverse vinblastine resistance in LLC-GA5-COL300 cells was examined (Fig. 6A). Progesterone increased the sensitivity of LLC-GA5-COL300 cells to vinblastine, whereas cortisol scarcely increased it, consisting with the effect of these steroid hormones on vinblastine accumulation and on vinblastine resistance in mouse
24251

Human P-glycoprotein as a Steroid Transporter

(A) Cortisol
(B) Aldosterone
(C) Dexamethasone
(D) Progesterone

FIG. 4. Transepithelial transport of 3H-labeled agents in wild-type LLC-PK1 (○, △) and LLC-GA5-COL300 (●, ▲) monolayers. Cells were cultured on filter-bottomed caps. Source side medium was replaced with medium containing 11 nM 3H-labeled cortisol (A), 12 nM 3H-labeled aldosterone (B), 25 nM 3H-labeled dexamethasone (C), and 12 nM 3H-labeled progesterone (D). Transepithelial transport from basal to apical surfaces (○, △) and from apical to basal surfaces (●, ▲) were measured at 1, 2, and 3 h. Net basal to apical transport was calculated by subtracting transport from apical to basal surfaces from transport from basal to apical surfaces at 3 h. Net basal to apical transport of cortisol was 2.6% in LLC-PK1 cells and 22.0% (19,700 dpm) in LLC-GA5-COL300 cells, resulting in a 8.3-fold increase; that of aldosterone was 4.5% in LLC-PK1 cells and 15.3% (9100 dpm) in LLC-GA5-COL300 cells, resulting in a 3.4-fold increase; that of dexamethasone was 4.7% in LLC-PK1 cells and 30.7% (28,000 dpm) in LLC-GA5-COL300 cells, resulting in a 6.5-fold increase; and that of progesterone was 5.8% in both cell lines.

FIG. 5. Effects of verapamil on steroids transport in LLC-GA5-COL300 cells. Transepithelial transport of 3H-labeled cortisol (A), aldosterone (B), and dexamethasone (C) was measured in the absence (○) or presence (●, ▲) of 20 μM verapamil. ○ and △, basal to apical transport; ● and ▲, apical to basal transport.

MDR cells (8). Progesterone had a small influence (<2-fold) on the IC50 for vinblastine in LLC-PK1 host cells (Fig. 6B). The concentration (50 μM) of progesterone and cortisol allowed >90% of normal cell growth in the absence of vinblastine. These results also indicate that progesterone has a high affinity for human P-glycoprotein whereas cortisol has a very low affinity for human P-glycoprotein.

DISCUSSION

In this paper, we showed that human P-glycoprotein transports cortisol and aldosterone, but not progesterone. Human
P-glycoprotein is expressed in the zona glomerulosa, zona fasciculata, and zona reticularis (3). Cortisol, a glucocorticoid, is the main steroid produced in the human adrenal gland, particularly in the zona fasciculata (26). Aldosterone is the main mineralocorticoid produced in the zona glomerulosa of the human adrenal gland (27). Results of transport experiments and the location of expression strongly suggest that P-glycoprotein aids in the secretion of cortisol and aldosterone out of cells in the human adrenal cortex.

During human pregnancy, estrogens and progesterone are produced in the fetoplacental unit, and metabolic intermediates move among the fetus, placenta, and mother. Because P-glycoprotein is expressed in the pregnant uterus and placenta (6), it may be important in transporting metabolic intermediates of estrogens and progesterone in the fetoplacental unit. Because an excess of progesterone inhibited the transepithelial transport of cortisol (Fig. 7), it may regulate the function of P-glycoprotein in the fetoplacental unit and adrenal cortex.

Cortisol is transported by human P-glycoprotein, although it scarcely inhibits azidopine photoaffinity labeling (Fig. 1B) or scarcely affects sensitivity to vinblastine (Fig. 6). Progesterone is not transported by human P-glycoprotein, although it inhibits azidopine photoaffinity labeling and increases the sensitivity of LLC-GA5-COL300 cells to vinblastine. These results suggest that a slight difference in the structure of steroids determines whether it is transported by P-glycoprotein and that agents that efficiently compete with azidopine photoaffinity labeling and vinblastine transport of P-glycoprotein are not necessarily transported by P-glycoprotein.

Safa et al. (28) reported that an amino acid substitution (Gly to Val) at position 185 of human P-glycoprotein increases the efficiency of P-glycoprotein in the efflux of colchicine, but decreases the binding of a photoactive colchicine analog. In contrast, the Gly to Val substitution decreases its efficiency in the efflux of vinblastine, but increases the binding of a photoactive vinblastine analog (28). These results suggest that efficiency in competing with azidopine photoaffinity labeling does not parallel the efficiency in the transport by P-glycoprotein. It is proposed that the Gly to Val substitution at position 185 affects not the initial binding site of P-glycoprotein, but another site, associated with the release of P-glycoprotein-bound drugs to the outside of the cell (28). Naito and Tsuuro (25) reported that progesterone has an apparent inhibitory constant similar to that of adriamycin for the ATP-dependent binding of vincristine to human MDR cells. Adriamycin is actively transported out of the cells by P-glycoprotein, whereas progesterone is not transported. These results suggest that the binding efficiency of a substrate is not the factor that determines whether P-glycoprotein transports it.

It has not been determined how P-glycoprotein recognizes many different hydrophobic molecules that share no obvious structural similarity and how P-glycoprotein actively transports them out of the cells. We are now examining which steroid metabolites are transported by human P-glycoprotein and which are not. These experiments may shed light on the structural specificity of substrates for P-glycoprotein to transport. Using this transepithelial transport system, we may not only be able to identify the physiological functions of human P-glycoprotein in more detail, but also find a clue to understand the mechanism through which substrates are recognized and transported by P-glycoprotein.

Acknowledgments—We thank Dr. Iwao Furusawa for electron microscopy photographs. We thank Drs. I. Pastan and M. M. Gottesman for reading the manuscript.

REFERENCES

1. Gottesman, M. M., and Pastan, I. (1988) J. Biol. Chem. 263, 12163-12166
2. Endo, J. F., and Ling, V. (1989) Annu. Rev. Biochem. 58, 137-171
3. Sugawara, I., Nakahara, M., Hamada, H., Tsuuro, T., and Mori, S. (1988) Cancer Res. 48, 4611-4614
4. Thiebaut, F., Tsuuro, T., Hamada, H., Gottesman, M. M., Pastan, I., and Willingham, M. C. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 7735-7738
5. Thiebaut, F., Tsuuro, T., Hamada, H., Gottesman, M. M., Pastan, I., and Willingham, M. C. (1989) J. Histochem. Cytochem. 37, 159-164
6. Arceci, R. J., Croop, J. M., Horwitz, S. B., and Howman, D. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 4350-4354
7. Yang, C. P. H., Cohen, D., Greenberger, L. M., Hsu, S. I. H., and Horwitz, S. B. (1989) J. Biol. Chem. 264, 10262-10266
8. Yang, C. P. H., DePinho, S. G., Greenberger, L. M., Arceci, R. J., and Horwitz, S. B. (1990) J. Biol. Chem. 264, 782-789
9. Naito, M., Yusa, K., and Tsuuro, T. (1989) Biochem. Biophys. Res. Commun. 158, 1066-1071
10. Quan, X., and Beck, W. T. (1990) J. Biol. Chem. 265, 18753-18756
11. Ueda, K., Clark, D. P., Chen, C., Roninson, I. B., Gottesman, M. M., and Pastan, I. (1987) J. Biol. Chem. 262, 509-508
12. Kane, S. E., Reinhard, D. H., Ford, C. M., Pastan, I., and Gottesman, M. M. (1989) Gene (Amst.) 84, 439-446
13. Kioka, N., Tsubota, J., Kakehi, Y., Komano, T., Gottesman, M. M., Pastan, I., and Ueda, K. (1989) Biochem. Biophys. Res. Commun. 162, 224-231
14. Graham, P. L., and vander Eh, A. J. (1970) Virology 45, 466-467
15. Kaufman, H. J., Murtha, P., Ingolia, D. E., Young, C. Y., and Kellens, R. E. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 3136-3140
16. Cornell, M. M., Gottesman, M. M., and Pastan, I. (1986) J. Biol. Chem. 261, 7921-7928
17. Safa, A. R., Glover, C. J., Sewell, J. L., Meyers, M. R., Biedler, J. L., and Pelased, R. L. (1987) J. Biol. Chem. 262, 7864-7868
18. Carmichael, J., DeGraff, W. G., Gazdar, A. F., Minna, J. D., and Mitchell, J. B. (1987) Cancer Res. 47, 936-942
19. Horio, M., Chiu, K. V., Currier, S. J., Goldenberg, S., Williams, C., Pastan, I., Gottesman, M. M., and Handler, J. (1989) J. Biol. Chem. 264, 14880-14884
20. Misfeldt, D. S., and Sanders, M. J. (1981) J. Membr. Biol. 59, 15-18
21. Fazli, C., Rosser, B., and Rohc-Ramal, F. (1988) Am. J. Physiol. 254, F341-F357
22. Fouza, A. R., Fauth, C., and Roch-Ramal, F. (1990) J. Pharmacol. Exp. Ther. 252, 299-295
23. Takayama, A., Okazaki, Y., Fukuda, K., Takano, M., Inui, K., and Hori, R. (1981) J. Pharmacol. Exp. Ther. 217, 209-214
24. Pastan, I., Gottesman, M. M., Ueda, K., Lovelace, E., Rutherford, A. V., and Willingham, M. C. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 4486-4490
25. Naito, M., and Tsuuro, T. (1989) Cancer Res. 49, 1452-1455
26. Nishikawa, T., and Scott, C. A. (1984) Endocrinology 114, 495-499
27. Tait, S. A. S., Scholzler, D., Okamoto, M., Flood, G., and Tait, J. F. (1970) Endocrinology 86, 309-381
28. Safa, A. R., Stern, R. K., Choi, K., Argesti, M., Tanai, I., Mehta, N. D., and Roninson, I. B. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 7225-7229