Targeted Disruption of the Mouse mdr1b Gene Reveals That Steroid Hormones Enhance mdr Gene Expression*

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To evaluate the role of P-glycoprotein in steroid secretion in adrenal cells, we have used gene targeting to introduce a null mutation into one allele of the mdr1b gene in mouse Y1 adrenal cells. Characterization of both the wild-type and the mutant cell lines revealed the following. 1) The expression of mdr1b is enhanced by steroid hormones, in a feedback regulatory mechanism. Inhibition of steroid biosynthesis by 2-aminogluthethimide blocks the adrenocorticotropic hormone (ACTH)-induced increase in mdr1b mRNA levels. 2) ACTH-stimulated steroid secretion is markedly decreased in the mutant cell line. This decreased steroid secretion in the mutant cells occurs despite an increase in the levels of mdr1b mRNA and P-glycoprotein. Kinetic analyses of vinblastine and daunomycin accumulation in both the wild-type and the mutant cell lines during ACTH-stimulated steroidogenesis show that in the mutant cells both drugs accumulated to higher levels than in Y1 cells, suggesting that the remaining mdr1b allele in the mutant cells is relatively inactive as an exporter of steroids, or that the targeted disruption of the mdr1b allele is associated with other changes in the mutant cells which block ACTH-stimulated steroid secretion.

The emergence of multidrug resistance in cultured cells has been associated with the overexpression of a small family of mdr genes encoding P-glycoproteins (Endicott and Ling, 1989; Gottesman and Pastan, 1993). The P-glycoproteins function in drug-resistant cells as energy-dependent, multidrug efflux pumps. The mdr gene family is composed of three members in rodents (mdrla, mdr1b, and mdr2) and two members in humans (MDR1 and MDR2). Transfection studies with the various mouse and human mdr genes have shown that the mouse genes, mdrla and mdr1b (also called md3 and mdr1, respectively) and the human MDR1 gene can confer the multidrug resistance phenotype to drug-sensitive cells, whereas the mdr2 genes cannot (Gros et al., 1986, 1988; Devault and Gros, 1990; Ueda et al., 1987).

In humans and mice the mdr genes are expressed in a tissue-specific manner (Croop et al., 1989). In mice the mdr1b gene is expressed mostly in the adrenal gland, kidney, pregnant uterus and placenta, whereas the mdr1la gene is expressed mainly in intestine and lung. Although P-glycoprotein is responsible for resistance to exogenously administered drugs, the normal physiological function of the P-glycoprotein family is unknown. The localization of P-glycoprotein in different normal tissues suggests that it may function as a transporter of endogenous cellular metabolites and naturally occurring toxic substances (Thiebaut et al., 1987). Several observations also suggest possible interactions between the multidrug transporter and steroids. The combination of estrogen and progesterone increases mdr1b expression in the secretory epithelium of the endometrium (Arceci et al., 1988, 1989). Progesterone, whose level is increased during pregnancy, is known to interact with P-glycoprotein (Yang et al., 1989). It was also shown that progesterone increases the activity of the mdr1b promoter via a response site located in the first exon of the gene (Pickart et al., 1993). Corticosterone appears to be a substrate for transport by P-glycoprotein (Wolf and Horwitz, 1992), and human MDR1 cDNA expressed in porcine LLC-PK1 cells is able to transport cortisol, aldosterone, and dexamethasone but not progesterone (Ueda et al., 1992). Also, cell lines with a high expression of P-glycoprotein display a reduced accumulation of cortisol and an ATP-dependent outward transport of the hormone (van Kalen et al., 1993). In mouse adrenocortical Y1 cells, steroid secretion is blocked by high concentrations of inhibitors of P-glycoprotein function (Chin et al., 1992). Also, wild-type levels of cAMP-dependent protein kinase are required for maintaining basal levels of mdr1b RNA in Y1 cells (Chin et al., 1992). Production of steroid hormones in the adrenal gland is regulated by adrenocorticotropic hormone (ACTH) which works through specific cell surface receptors to activate adenylate cyclase and increase intracellular cAMP (Orme-Johnson, 1990). Most of the effects of ACTH are thought to occur via cAMP through the action of cAMP-dependent protein kinase. The acute response to ACTH stimulation results in a rapid increase in steroid hormone production presumably because of increased availability of cholesterol precursor. The Y1 mouse adrenocortical tumor cell line is functionally similar to normal adrenal cells; it synthesizes steroid hormones from cholesterol in response to ACTH stimulation (Kowal, 1970). The Y1 cell line therefore provides a useful model system for

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1 The abbreviations used are: ACTH, adrenocorticotropic; 2-AG, 2-aminogluthethimide; 8-Br-cAMP, 8-bromo-cAMP; kb, kilobase(s).
Adrenal mdr1 Expression and Function

In this report we describe the use of gene targeting in the Y1 steroid-secreting epithelial cells to introduce a null mutation into one allele of the mdr1b gene. Characterization of both the wild-type and the mutant cell lines reveals a feedback regulation mechanism involving P-glycoprotein and steroid hormones.

**EXPERIMENTAL PROCEDURES**

**Cell Culture**—The Y1 adrenal cell line was cultured in minimal essential medium-a (Life Technologies, Inc.) supplemented with 15% (v/v) heat-inactivated horse serum, 2.5% (v/v) newborn calf serum, and 2 mM L-glutamine. The medium for the mutant cell line also contained 4G18 (0.5 mg/ml).

**Electroporation**—Cells were trypsinized about 2 h after refeeding, washed twice, and resuspended at a concentration of 10^7 cells/ml in phosphate-buffered saline. Then the cells were plated on ice with 25 μg of linearized DNA (with Sall) for 10 min and electroporated at room temperature at 250 mA, 680 microfarads using a Bio-Rad Gene Pulser. After electroporation, the cells were incubated on ice for 10 min and then plated in regular medium. The cultures were allowed to recover for 36 h prior to the addition of 4G18 (0.5 mg/ml) and gancyclovir (5 μM).

**RNA Analysis**—1.5 × 10^6 cells were plated in a 100-mm dish in regular medium. The next day, about 2 h after refeeding, the cells were incubated overnight in medium with or without ACTH and 2-aminogluthemide (2-AG). Total RNA extraction from cells, transfer to nitrocellulose, and hybridization have been described previously (Fojo et al., 1987; Goldstein et al., 1989; Chin et al., 1992).

**Immuno precipitation—**P-glycoprotein was immunoprecipitated from cells labeled with [3H]methionine and [35S] cysteine (Tran3'S-label; ICN) in 5 ml of methionine, cysteine-free medium. At approximately 18 h the cells were washed and harvested as described previously (Chin et al., 1992). Aliquots of supernatants were reacted overnight with rabbit polyclonal antisera Rm raised against peptide 110-117 of the P-glycoprotein (Dr. Lee Greenberger). The complexes were precipitated with protein A-Sepharose (Pharmacia LKB Biotechnology Inc.) as described in Tanaka et al. (1990) and loaded on a sodium dodecyl sulfate-6% polyacrylamide gel.

**Measurement of Secreted Steroids—**Fluorogenic steroids were measured using a modification of the method of Kowal and Fiedler (1968). Seven × 10^6 cells were plated in a 60-mm dish in regular medium. The next day, 2 h after refeeding, the cells were incubated in 1.3 ml of medium with or without ACTH, 8-Br-cAMP or both ACTH and 2-AG (2-AG was added 3 h prior to the addition of ACTH). At 18 h, after centrifugation, 500 μl of culture medium was extracted for 15 min with 0.9 ml of methylene chloride in a microcentrifuge tube. The organic phase was then extracted overnight in medium with or without ACTH and 2-aminogluthemide. The mixture of steroid hormones was measured using the method of Bradford (1976). The next day, about 2 h after refeeding, the cells were incubated in 1.3 ml of medium with or without ACTH, 8-Br-cAMP or both ACTH and 2-AG (2-AG was added 3 h prior to the addition of ACTH). At 18 h, after centrifugation, 500 μl of culture medium was extracted for 15 min with 0.9 ml of methylene chloride in a microcentrifuge tube. The organic phase was then extracted overnight in medium with or without ACTH and 2-aminogluthemide.

**Uptake Assays—**Five × 10^6 cells were plated in a 35-mm culture dish in regular medium. The next day, 2 h after refeeding, the cells were incubated in 0.7 ml of medium with or without ACTH for 18 h. At time zero of the experiment 1 ml of phosphate-buffered saline containing glucose (5 mM) and either vinblastine (0.1 μg/ml cold vinblastine and 0.25 μCi/ml [3H]vinblastine sulfate; Amersham Corp.) or daunomycin (1 ng/ml cold daunomycin and 1 μCi of [3H] daunomycin; Du Pont NEN) was added to each dish, and the dishes were incubated at room temperature for the indicated times. The experiment was terminated by three rapid washes of the cells with ice-cold phosphate-buffered saline. The cells were then taken for liquid scintillation counting as described in Koren et al. (1979).

**RESULTS**

**ACTH-stimulated Steroidogenesis Affects mdr1b Expression—**To study the effect of steroid hormones on mdr1b expression, total RNA was prepared from the wild-type Y1 cell line and subjected to slot blot analysis. The mdr1b mRNA levels were measured with and without prior treatment of the cells with ACTH. The results shown in Fig. 1 (left) reveal a 4–5-fold increase in the level of mdr1b mRNA in response to ACTH in the Y1 cell line. To show that the increase in the mRNA level is caused by steroid synthesis, the cells were treated with both ACTH and 2-AG, an inhibitor of the first reaction of steroid hormone synthesis. As can be seen in Fig. 1, this steroid biosynthesis inhibition blocked the ACTH-induced increase in mdr1b mRNA levels in a dose-dependent manner. The level of mdr1b-P-glycoprotein in Y1 cells was determined by immuno precipitation of labeled cell extracts with and without prior treatment of the cells with ACTH. An increase of 2–3-fold in the level of mdr1b-P-glycoprotein was detected in response to ACTH in Y1 cells (not shown). These results indicate that steroids enhance the level of the mdr1b-P-glycoprotein transporter.

**Disruption of the mdr1b Locus by Gene Targeting—**To address the question of the involvement of P-glycoprotein in steroid secretion in Y1 cells, we mutated the mdr1b allele, using a replacement vector similar to that designed by Mansour and co-workers (Mansour et al., 1988; Chauhan and Gottesman, 1992). The vector contains two selectable markers to allow for both positive and negative selections. A neo expression cassette, which serves to select vector-transformed cells and to mutate the gene, was inserted in exon 19 embedded in a 4.7-kb genomic fragment of mdr1b (Fig. 2A). Outside of the mdr fragment, two herpes simplex thymidine kinase genes were placed, one at either end. The thymidine genes confer sensitivity to purine analogs such as gancyclovir and were used to select against random integration events. The mdr1b homologous recombination vector was linearized and transfected into Y1 cells by electroporation. To avoid polyplody, the cells were subcloned prior to transfection, and a diploid copy of chromosomes was confirmed in the Y1 subclone used for these studies. In addition, the diploid copy number of the mdr1 gene was confirmed by Southern blot analyses, using different diploid mouse embryonic cell lines for reference (not shown).

**G418- and gancyclovir-resistant colonies were detected after 11–12 days, clonally expanded, and screened for homologous recombination by Southern blot analysis. Genomic DNA from approximately 200 different clones was digested with EcoRI and hybridized to an external probe corresponding to sequences 5′ of the mdr1b sequences sequenced in the targeting vector (Fig. 2; PstI probe). One clone A1 showed the expected fragment predicted following homologous recombination, in addition to the fragment generated by the normal allele (Fig. 2B; 5.2 and 3.7 kb, respectively). To examine the A1 clone further, DNA digests were hybridized to a neo cDNA probe (Fig. 2B) and an mdr1b cDNA probe (not shown). As can be seen in Fig. 2B, hybridization to the neo probe detected only the predicted bands of 3.7 and 2.6 kb, indicating that the vector was inserted at a single insertion site in the mdr1b gene in the A1 clone. Thus, one allele of the mdr1b gene has been disrupted.

**Analysis of Steroid Secretion in Y1 and A1 Cells—**To determine whether the disruption of one allele of the mdr1b gene affected steroid secretion, Y1 and A1 cells were treated with either ACTH or the cAMP analog 8-Br-cAMP, both known to stimulate steroidogenesis in these cells. The secretion level of fluorogenic steroids was measured by using the assay of Kowal and Fiedler (1968). The results depicted in Fig. 3 show that under basal conditions, steroid secretion is somewhat reduced in A1 cell lines (the original mutant parent and one...
of its subclones) compared with the Y1 parent. Moreover, although the parent cells respond to ACTH or 8-Br-cAMP stimulation with a 2.5- to 3.5-fold increase in steroid secretion, the mutant cells show almost no increase in steroid secretion under these conditions.

The lack of steroid secretion in response to both ACTH and 8-Br-cAMP stimulation in the mutant could be caused by inactivation of the \textit{mdr1}\textsubscript{b} gene product or could reflect the existence of a secondary mutation in either the steroid biosynthesis pathway or in the cAMP response pathway. It was shown previously that cAMP-dependent protein kinase mutants of Y1 cells (Kin) exhibit reduced steroidogenesis in response to 8-Br-cAMP or ACTH (Rae \textit{et al.}, 1979). Therefore, protein kinase activity was measured in dialyzed extracts of Y1 and A1 cells, following activation by cAMP. The activity was determined with and without prior treatment of the cells with ACTH. A similar, unaltered cAMP-dependent protein kinase activity was measured in both cell lines under basal conditions, and a similar increase in protein kinase activity was seen in both cell lines after ACTH treatment (not shown). In addition, media from both A1 and Y1 cell lines contain a basal level of steroids. The addition of 2-AG, known to inhibit the first reaction of steroid hormone biosynthesis, resulted in a complete elimination of steroids from the media of the mutant A1 as well as of the wild-type Y1 (Fig. 3), suggesting that the reduction in steroid secretion in stimulated A1 cells is probably not caused by a defect in steroid biosynthesis.

\textbf{Expression of \textit{mdr1}\textsubscript{b} Gene in Y1 and A1 Cells}—To determine the level of expression of the mutated allele and the effect of this mutation on the expression level of the remaining intact allele, total RNA was prepared from parent (Y1) and mutant (A1) cell lines soon after the homologous recombinant clone was detected. The RNA was subjected to Northern blot analysis. Hybridization with a cDNA probe specific for \textit{mdr1}\textsubscript{b} revealed an mRNA of approximately 5 kb in both cell lines as well as in the control mouse adrenal tissue (Fig. 4A).
Surprisingly, the mRNA level observed with the mutant A1 was approximately 30-fold higher than that of the parent Y1. A quantitative slot blot analysis of total RNA from these cell lines confirmed that the A1 mRNA level is elevated, and it reached the high level observed in normal mouse adrenal tissues (Fig. 4B). A similar slot blot analysis of total RNA from many different subclones of Y1, as well as neo8 clones in which the targeting vector integrated randomly, did not show this marked increase in mRNA expression (Fig. 4B and not shown), indicating that the markedly elevated expression of mdrlb RNA emerged as a result of the insertional mutagenesis.

Hybridization of the Northern blot with a neo-specific probe revealed an approximately 1-kb mRNA, demonstrating that the neo mRNA is transcribed from its own promoter (Fig. 4A, right panel). The full-length mdr mRNA did not hybridize with the neo probe, as would be expected from an mdrlb-neo fused transcript, indicating that the high levels of mdrlb mRNA originated solely from the remaining, intact allele of the gene. The additional, larger bands observed with the neo-specific probe suggest that the fused transcript is made; however, it is susceptible to degradation. Hybridization of the slot blot with a cDNA probe specific to the mdrla gene showed almost no difference in the mdrla mRNA levels in the cell lines analyzed (Fig. 4B), demonstrating that the large increase in the mRNA level is specific for mdrlb mRNA and that the extinction of one mdrlb allele had no effect on the level of expression of the mdrla homolog.

The level of mdrlb-P-glycoprotein in Y1 and A1 cells was determined by immunoprecipitation of labeled cell extracts using a rabbit polyclonal antisera raised against a peptide specific to mdrlb. The results depicted in Fig. 4C show a large increase in the level of P-glycoprotein in the A1 mutant, indicating that the increase in mRNA level resulted in a comparable increase in protein level. No P-glycoprotein was detected when a nonimmune serum was used or when the antisera was used in the presence of the mdrlb peptide. After the initial characterization of the clone, the expression level of mdrlb in A1 cells decreased somewhat with passage in tissue culture. The rest of the experiments were done while the mutant cells retained an average of 3–5-fold increased expression of mdrlb-P-glycoprotein compared with the wild-type Y1 cell line (see below).

The levels of A1 mdrlb mRNA were also measured with and without prior treatment of the cells with ACTH. The results shown in Fig. 1 (middle) reveal a 4–5-fold increase in mdrlb mRNA levels in A1, in response to ACTH. In addition, as observed for the wild-type (Y1) cell line, 2-AG, an inhibitor of steroid biosynthesis, blocked the ACTH-induced increase in mdrlb mRNA in the mutant (A1) (Fig. 1, middle), implying that this increase was mediated by steroids in the mutant cell line. Hybridization of the slot blot carrying the A1 RNA with a neo probe after removal of the mdrlb probe showed no difference in the neo mRNA level in response to either ACTH or 2-AG, demonstrating that the adrenal steroidogenesis affects mdr expression in a specific manner (Fig. 1, right). The finding that the A1 mutant lacks the ability to secrete steroids

![Fig. 3. Steroid secretion in Y1 and A1 cells.](image)

**Fig. 3.** Steroid secretion in Y1 and A1 cells. Cells were incubated for 18 h in ACTH (10^{-7} M), 8-Br-cAMP (1 mM), and 2-AG (350 μM) as indicated on the figure. At the end of the incubation, the media were collected and extracted, and steroids were measured as described under "Experimental Procedures." All values represent the average of five experiments. A1-3-1 is one of the subclones of the original mutant parent A1.

![Fig. 4. Expression of mdrlb mRNA and protein in Y1 and A1 cells. Panel A, RNA from Y1 cells (10 μg), A1 cells (10 μg), and mouse adrenal tissue (3.5 μg) was subjected to Northern blot analysis using either an mdrlb-specific probe (Chin et al., 1992) or a Neo probe. The positions of the approximately 5-kb (mdrlb) and 1-kb (neo) messages are indicated by the upper and the lower arrows, respectively. Panel B, total RNA from Y1, Y1-Neo (randomly integrated-control cell line), A1 cells, and adrenal tissue was subjected to slot blot analysis using mdr1b- and mdrla-specific probes. Comparable RNA loading was confirmed using γ-actin probe. Panel C, immunoprecipitation of mdr1b from Y1 and A1 cells using a rabbit polyclonal antisera raised against an mdr1b-specific peptide (Ab-Rm and peptide 110 are a gift from Dr. Lee Greenberger). Third lane from left, A1 extract with nonimmune serum; far right lane, immunoprecipitation of mdr1b from A1 extract using Rm serum and 4 μg/ml peptide 110. The arrow indicates the position of P-glycoprotein.
in response to ACTH stimulation while its remaining allele expresses high levels of mdr1b RNA and protein suggests either that the remaining P-glycoprotein is not as active as the P-glycoprotein in the parent cell line or that the mutant carries a defect in P-glycoprotein (or at another site) which indirectly diminishes ACTH-stimulated steroid secretion.

Drug Uptake in Y1 and A1 Cells—One well recognized function of mdr1b is to prevent accumulation of drugs within cells. To test the activity of the remaining mdr1b allele in the presence of ACTH, a series of uptake studies of two mdr substrates were performed. Fig. 5A depicts the uptake of vinblastine and Fig. 5B of daunomycin into Y1 (wild-type) and A1 (mutant) cells. The cells were incubated in complete medium with or without ACTH for 18 h. At time zero, daunomycin or vinblastine was added, and the cells were incubated in phosphate-buffered saline containing glucose, at room temperature. Then, at the indicated times, the medium was aspirated, and the cells were washed and counted. A 2-fold increase in both daunomycin and vinblastine accumulation was observed with Y1 cells when engaged in steroid secretion after ACTH treatment. These results further emphasize that mdr1b-P-glycoprotein has a role in steroid secretion and that steroids synthesized inside the cell and drugs that entered the cell by diffusion can compete for the same efflux activity of P-glycoprotein. When A1 cells were treated with ACTH, both drugs accumulated to higher levels (4-6-fold) than in Y1 cells, indicating that mdr1b-P-glycoprotein in A1 cells is defective in drug export in the presence of ACTH-stimulated steroidogenesis. The interaction between P-glycoprotein and steroid secretion is more pronounced in A1 cells, possibly because the remaining P-glycoprotein in these cells is less active as an exporter of steroids, so that the internal level of the competing steroids is higher than in Y1 cells. These steroids inhibit the ability of P-glycoprotein to pump out vinblastine and daunomycin.

**DISCUSSION**

To evaluate the role of the mdr1b gene in steroid secretion in adrenal cells, we have used gene targeting to introduce a null mutation into one allele of the mdr1b gene in mouse Y1 adrenal cells. Characterization of both the wild-type and the mutant cell lines revealed a feedback regulatory mechanism involving P-glycoprotein, encoded by the mouse mdr1b gene, and steroid hormones. We find that an increase in steroid biosynthesis stimulated by ACTH results in an increase in the level of mdr1b mRNA in Y1 cells. However, despite the elevated mRNA levels, the ability of P-glycoprotein to export both daunomycin and vinblastine decreases when the cells are engaged in steroid secretion. We conclude that endogenous steroids can act as competitive inhibitors for the drug transport function of P-glycoprotein. In addition, an increase in the intracellular level of steroids apparently causes an increase in the level of the transporter mdr1b mRNA and its product, P-glycoprotein, suggesting a feedback mechanism for the regulation of mdr1b gene expression.

**Enhancement of mdr1b Expression by Steroid Hormones—** Analysis of mdr1b mRNA levels upon ACTH-stimulated steroidogenesis revealed that regulation of mdr1b mRNA in the Y1 adrenal cell line is mediated by steroid hormones; a 4-5-fold increase in mdr1b mRNA levels is observed in response to ACTH. No increase in the mRNA level is detected when steroid biosynthesis is inhibited by the addition of 2-Ag. Previous reports of sequence comparisons of the 5'-flanking regions of the mouse mdr1b and mdr1a genes have defined a putative glucocorticoid response element in the promoter of the mdr1b gene (Cohen et al., 1991). Recently, an additional progesterone response element was identified in the first untranslated exon of the mdr1b gene (Piekarz et al., 1993). Both sites are able to confer responsiveness to the nonresponsive thymidine kinase-chloramphenicol acetyltransferase vector upon interaction with the A form of the progesterone receptor (Piekarz et al., 1993). In view of the results reported here, it is conceivable that transcription of mdr1b in adrenal cells is enhanced by steroid hormones directly, through the binding of the steroid hormone receptor to either one or both of the glucocorticoid response elements.

**Steroid Hormone Secretion Mediated by mdr1b-P-glycoprotein—** The potential involvement of P-glycoprotein in steroid secretion was examined by studying drug uptake in the presence of ACTH-stimulated steroidogenesis. We found that the efflux activity of P-glycoprotein decreases when the cells are engaged in steroid secretion, despite an increase in P-glycoprotein mRNA. This result agrees with the finding that steroid secretion in Y1 cells is blocked by high concentrations of known inhibitors of the transporter (Chin et al., 1992) and is consistent with the hypothesis that steroids are substrates for P-glycoprotein and can act as competitive inhibitors for its drug transport function (Wolf and Horwitz, 1992).

Gene targeting was used to generate an experimental model system for evaluating the role of P-glycoprotein in adrenal cell function. We found that extinction of one allele of the
The mdr1b gene in mouse Y1 cells by insertional mutagenesis caused an increase in the expression of the remaining intact allele. We postulate that the initial increase in expression is caused by the feedback regulatory mechanism described above.

The finding of the decreased ACTH-stimulated steroid secretion and decreased drug efflux despite a higher level of P-glycoprotein mRNA and protein in the A1 strain was unexpected. These observations suggest that the transport activity of the remaining P-glycoprotein may be decreased. The relative contribution to expression of the two alleles of the mdr1b gene is unknown. It is possible that in these cells the second allele does not encode a P-glycoprotein as active as the one that underwent the insertional mutagenesis. However, we have used PCR to clone overlapping cDNAs from P-glycoprotein mRNA derived from mutant A1 cells and have found no mutations in these cDNAs.

Alternatively, a protein fragment that is made, as a result of the insertional mutagenesis in exon 19 located in the 3' half of the gene, may inhibit the steroid export activity of the intact P-glycoprotein in a trans-dominant manner. This putative protein fragment might not be detected by the antiserum used in this study, since the antisera was raised against a peptide located in the vicinity of the insertion site. Finally, it is possible that the mutant A1 carries a second mutation which directly blocks P-glycoprotein function.

Understanding the regulation of the mdr gene family and the factors that control the differential expression of its members may play an important role in defining therapeutic options for the treatment of malignancy and in predicting the effect of pharmacologic ablation of mdr gene function during cancer treatment.

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