Analysis of MDR1 Expression in Normal and Malignant Endometrium by Reverse Transcription-Polymerase Chain Reaction and Immunohistochemistry

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ABSTRACT
The purpose of this study was to quantitate the expression of human MDR1 mRNA levels in normal endometrium and in endometrial carcinoma and to determine the association of MDR1 mRNA levels with prognostic indicators. Endometrial samples from 43 postmenopausal patients with endometrial carcinoma and 38 patients (controls) with benign disease undergoing hysterectomy were snap-frozen. MDR1 levels were determined by quantitative reverse transcription-PCR (RT-PCR) and compared to sensitive and resistant cell lines. Immunohistochemistry was done with MM4.17, an anti-MDR1 antibody, on paraffin sections, and the results were compared to those obtained from RT-PCR. Data was analyzed using the Kruskal-Wallis and Bonferroni tests, setting the P value at 0.05. In both postmenopausal endometrial tissue and tumors, MDR1 expression was localized to the epithelial cell layer. Comparison of immunohistochemistry and RT-PCR results demonstrated a correlation of 80%. In control patients, MDR1 expression was significantly higher in postmenopausal endometrium (n = 15) than in the proliferative premenopausal endometrium (n = 15; P = 0.0024). MDR1 expression in all tumors was lower than that measured in the postmenopausal controls. Between each tumor group, there was no significant difference in the MDR1 levels observed. MDR1 expression was significantly lower in patients with high nuclear grade (n = 18) tumors when compared to patients with low nuclear grade (n = 14; P = 0.04) tumors. Comparison of MDR1 levels with multiple prognostic indicators for endometrial cancer was only significant for nuclear grade. The data indicate that MDR1 expression is not a major component of the drug resistance observed in primary endometrial tumors.

INTRODUCTION
The development of resistance to chemotherapy is one of the major obstacles to the successful treatment of many malignancies. In general, tumors treated with a single agent develop alterations in the drug target that result in clinical resistance. However, some tumors become resistant to a broad spectrum of antitumor drugs after treatment with only a single agent; this phenomenon is known as MDR3 (1, 2). Only certain drugs, particularly hydrophobic natural products such as Adriamycin, Taxol, and vinblastine, induce MDR. The development of MDR has been extensively studied, using highly drug-resistant cultured cell lines as model systems. In characterizing these cell lines, it was observed that the highly resistant cell lines accumulated less drug intracellularly compared to the parental cell lines (3, 4). In addition, these cells overproduced a membrane glycoprotein, P-gp, whose level of expression correlates with the observed degree of resistance (5).

P-gp is encoded by a small gene family, MDR1 (6) and MDR2 (7, 8) in humans and mdr1a, mdr1b, and mdr2 in mice (9, 10). MDR1, mdr1a, and mdr1b genes, which confer drug resistance to a sensitive cell, are considered Class I genes, whereas MDR2 and mdr2, which do not confer drug resistance, are classified as Class II genes. It is believed that P-gp functions as an energy-dependent efflux pump, thus maintaining the intracellular drug concentration below cytotoxic levels (11).

Although P-gp was originally described in drug-resistant cells, it is now known that it is present in many normal tissues in both mouse and human. There is a high level of P-gp expression in adrenal cortical cells, the kidney brush border, the canalicular surface of hepatocytes, the small and large intestinal mucosal cells, and the pancreas (12–15). In mouse, the expression is isomeric-specific, with mdr1a highly expressed in the intestine, liver, and lung and mdr1b expressed at high levels in the adrenals, kidney, and, interestingly, in the uterus during pregnancy (16). However, the function of P-gp in normal tissues has not yet been elucidated. From studies in transgenic mice, it has been suggested that mdr1a functions in the blood-brain barrier to protect against environmental toxins (17) and that mdr2 functions in the liver to transport phospholipids into the bile (18).

Many tumors that originate from tissues with high levels of MDR1 expression are difficult to treat using chemotherapeutic agents. This has led to the hypothesis that certain tumors are intrinsically resistant, whereas others acquire resistance only after exposure to drug (19–23). MDR1 mRNA levels in tumors and cell lines seem to correlate with the observed degree of

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The abbreviations used are: MDR, multidrug resistance; RT, reverse transcription; P-gp, P-glycoprotein.
resistance (24), and increased expression of MDR1 is thought to be responsible for treatment failure in some tumors (25–27). MDR1 gene expression also has been implicated as a marker of tumor progression and aggressiveness. It would be beneficial to accurately predict whether a tumor will respond to chemotherapy before treatment. To this end, many tumor types have been evaluated for MDR1 mRNA and P-gp expression by RT-PCR and immunohistochemistry, respectively, to determine the correlation, if any, between these levels and patient outcome. To date, only in neuroblastoma, childhood sarcoma, and certain leukemias have the levels of MDR been predictive of poor outcome in these retrospective studies (25, 28).

In the mouse, mdr1b is highly expressed in the endometrium during pregnancy. Expression is restricted to the secretory columnar epithelium, which forms the endometrium (29, 30). Very little is known about the expression of MDR1 mRNA in the human uterus. Because the human and mouse genes are structurally and functionally similar and share almost identical tissue distribution, it is anticipated that MDR1 is also expressed in the human uterus. Retrospective studies using immunohistochemistry have demonstrated that MDR1 is expressed in tumors derived from the uterus (31–33) and in the normal uterus during pregnancy (34).

To fully characterize the expression of MDR1 in the human uterus, we have analyzed biopsy samples from benign endometrium as well as from tumors derived from endometrium by quantitative RT-PCR and immunohistochemistry. We have determined the levels of MDR1 in benign tissue from both premenopausal and postmenopausal endometrium. In addition, we have correlated expression of MDR1 mRNA with estrogen and progesterone receptor levels, stage and grade of the tumor, and other established prognostic indicators (35).

MATERIALS AND METHODS

Cell Lines and Tissue Procurement

The project was approved by the Internal Review Board at the Montefiore Medical Center. Samples were obtained over an 18-month period from a total of 91 patients, including 53 patients with endometrial hyperplasia or endometrial carcinoma undergoing hysterectomy and surgical staging and 38 pre- and postmenopausal patients with benign pathological conditions undergoing hysterectomy at the Weiler Division of Albert Einstein College of Medicine and Montefiore Medical Center. The endometrial tissue was divided into two sections; one was snap-frozen in liquid nitrogen and pulverized. Six ml of denaturing solution [4 M guanidinium thiocyanate, 25 mM sodium citrate (pH 7), 0.5% N-lauroylsarcosine, and 0.1 M 2-mercaptoethanol] were added. The mixture was extracted with 6 ml of water-saturated phenol and 1.2 ml of chloroform-isoamyl alcohol (49:1) and incubated on ice for 15 min. After centrifugation for 20 min at 4°C in a Beckman J-6B centrifuge at 2000 × g (2800 rpm), the aqueous-phase solution was precipitated with an equal volume of isopropanol in a −20°C freezer and centrifuged. The RNA pellet was resuspended in 75% ethanol, centrifuged at 2000 × g, and air-dried for at least 15 min. The pellet was resuspended in 60 μl of diethylpropylcarbonate-treated water. Approximately 1 μg of RNA was then used for RT.

RT of RNA. The RT-PCR quantitative procedure used is the method of Horikoshi et al. (37) with minor modifications. Briefly, for RT, 1 μg of RNA was added to 20 μl of 5× transcription buffer [250 mM Tris-HCl (pH 8.3), 375 mM KCl, and 15 mM MgCl2], 10 μl of 100 mM DTT, 10 μl of 10 mM deoxynucleotide triphosphate solution (10 mM final concentration of dATP, dCTP, dGTP, and dTTP, Pharmacia), 2.5 μl of 3 mg/ml of BSA, 0.5 μl of random hexamers [50 optical density units dissolved in 0.55 ml of 10 mM Tris-HCl (pH 7.5) and 1 mM EDTA], 2.0 μl RNAguard (Pharmacia), and 3 μl Moloney murine leukemia virus reverse transcriptase (Life Technologies, Inc.). The mixture was incubated in the thermocycler at 26°C for 8 min, 42°C for 90 min, and, finally, 95°C for 5 min. The cDNA synthesized was stored at −70°C.

PCR Primers for Reference and Target Genes. The PCR primers were synthesized by the Albert Einstein College of Medicine Oligonucleotide Facility. The primers were designed to span two adjacent exons to detect genomic DNA and amplify a region of 100–200 bases. Each 5’ primer also contained the T7 promoter sequence (the first 17 bases) followed by the transcription initiation sequence or a clamping sequence (the next 5–6 bases), which functioned to improve the yield of the transcription product. The exact sequence of this promoter is TAA TAC GAC TCA ATA TA (marked T7 in the sequences below). The initiation sequence is listed in quotation marks. The other sequences are: (a) 5’ T7-‘GGGAGA[GCGGGAATCTCGGTG]-GACTT 3’ (sense primer; bases 656–677) and 5’ GATGGAGTTGAGGTAGTTCGGT 3’ (antisense primer; bases 864–887) are the primer sequences for β-actin. The fragment generated is 232 bases long; and (b) 5’ T7-‘GGGA[C]CATT-CATTTCAATAGCAG 3’ (sense primer; bases 3020–3039) and 5’ GTTCAAACTTTGCTGCTGA 3’ (antisense primer; bases 3157–3176) are the primer sequences for MDR1. The fragment generated is 157 bases long.

PCR Reactions. A Perkin-Elmer Cetus 9600 thermocycler was used for the PCR reactions. The cDNA was diluted serially from 0 to 1:109. Ten μl of each dilution of cDNA were added to 2.5 μl of 10× Taq buffer [500 mM KCl and 100 mM Tris-HCl (pH 8.3)], 3.75 μl of 12.5 mM MgCl2, 0.5 μl of a 10 mM deoxynucleotide triphosphate solution (same as for RT), 1 μl each of the 5’ and 3’ primers at 12.5 pmol/μl, 10 μl of the cDNA solution, and 4.25 μl of 10 mM Tris-HCL. These tubes were heated for 5 min at 95°C in the thermocycler. Taq polymerase (Perkin Elmer Cetus) was diluted at 1:16, and 2 μl of the enzyme were then added to each tube. The PCR protocol was...
93.5°C for 1 min, 55°C for 1 min, and 72°C for 1 min for 30 cycles and 7 min at 72°C with storage at 4°C.

**T7 Polymerase Transcription Reaction.** Twenty μl of solution A (14.6 μl of diethylpropylcarbonate-treated water, 2.5 μl of 10× transcription buffer, 2.5 μl of 10 mM ribonucleotide solution, 0.25 μl of 1 mM DTT, 0.15 μl of RNaseA, 0.1 μl of 0.5 mM spermidine, and 0.25 μl of [α-32P]CTP at 300 Ci/mmol) were added to each tube containing 3.3 μl of each PCR product. The reaction was started by adding 1.67 μl of T7 RNA polymerase (EpiCenter), incubated for 1 h at 37°C. A total of 0.80 μl of 0.5 mM EDTA was added to stop the reaction.

**Gel Electrophoresis.** Twenty μl of the gel loading buffer (10 μl urea and 0.02% bromphenol blue) were added to each sample and incubated at 90°C for 10 min. Each sample was electrophoresed on a 6% denaturing polyacrylamide gel with 93.5°C for 1 mm, 55°C for 1 mm, and 72°C for 1 mm for 30 cycles and 7 min at 72°C with storage at 4°C.

**Immunohistochemistry Technique.** Paraffin-embedded sections were immunostained following the procedure in the Vectastain ABC kit with minor modification. Briefly, after dewaxing in Hemo-De (Fisher) and rehydrating in ethanol dilution series, the slides were incubated with 1% hydrogen peroxide in methanol for 30 min and pretreated with 10% normal goat serum and 20% normal horse serum for 30 and 40 min, respectively. Incubation with MM4.17, an IgG2α,K monoclonal antibody to the fourth extracellular domain of MDRI, was done at a concentration of 5.3 μl/ml for 18 h at 4°C (49). MM4.17 was a generous gift from Dr. M. Cianfraglia (Laboratorio di Immunologia, Rome, Italy). The samples were then immersed in 5 μg/ml of biotinylated horse anti-mouse IgG at room temperature for 30 min, followed by further incubation with an avidin and biotinylated horseradish peroxidase complex at room temperature for 45 min. The slides were developed in 3-3′ diaminobenzidine tetrahydrochloride containing 0.022% hydrogen peroxide, counterstained with hematoxylin, and dehydrated and mounted. The slides were washed with 0.1% saponin in sodium PBS [10 mM PBS (pH 7.5) and 200 mM NaCl] between each step, and 20% normal horse serum in 0.1% saponin/PBS buffer was used for all of the dilutions.

Paraformaldehyde-fixed cell preparations from a HeLa cell line transfected with mouse mdr1b were used to demonstrate the specificity of MM4.17 to human MDRI-encoded P-gp. As a negative control, mouse IgG (10 μg/ml) was used instead of the primary antibody. Acetone-fixed cytospin cell preparations of a vinblastine-resistant human ovarian tumor cell line (SKVLB) and a tumor sample with a high RT-PCR score were used as positive controls.

**Quantitation.** The intensity, percentage, and pattern of staining for each sample slide were recorded separately, and the results were summarized to yield a single score. The slides were reviewed and scored by a single pathologist (H-J. S.) who did not know the RT-PCR results. Two different staining patterns of P-gp expression were recorded: (a) cytoplasmic staining pattern as noted by dense focal perinuclear staining and a diffuse staining pattern; and (b) a membrane staining pattern as noted by a cell plasma membrane and luminal surface staining pattern.

**RESULTS**

The quantitative PCR methodology used in this study has been well documented by Horikoshi et al. (37). A schematic of the experimental procedures is shown in Fig. 1. A modification of RT-PCR methodology was chosen to determine the levels of MDRI expression in biopsy samples of benign and malignant endometrial samples. RNA was extracted from the tumor and converted into cDNA. The cDNA was serially diluted and amplified by PCR with primers for the gene of interest and for a gene, usually actin or β-microglobulin, that was used as an internal standard. Each reaction was done in a separate tube. It is assumed that the internal standard is produced universally and equally in the tissue samples (38). A linear relationship exists between the initial amount of cDNA and the final amount of PCR product. The 5′ primer of each set was modified to include the T7 phage promoter, allowing for subsequent transcription with T7 polymerase. After PCR was performed, the samples were transcribed into RNA using a radiolabeled CTP and analyzed by PAGE. The inclusion of the transcription step increased the sensitivity almost 500-fold and also allowed for accurate quantitation. The transcription products were excised and quantitated by scintillation counting, and the relative amount of MDRI as compared to actin was calculated.

In general, error is most often introduced in the initial quantitation of tissue RNA. By using this methodology, it is not necessary to know how much RNA is initially present. The level of the gene of interest is compared to an internal standard, and a relative value is calculated. In addition, our study used two independent means to verify the MDRI levels; each tissue block was divided in half, one section for PCR and the other for immunohistochemistry.

Representative autoradiograms of the MDRI and β-actin PCR products from a tumor sample and from drug-resistant cell lines are shown in Fig. 2. For each sample, the actual autoradiogram along with the quantitative analysis and the ratio of MDRI to actin is included. The results indicate that MDRI expression in this tumor sample is similar to MDRI expression in the low-resistance cell line. The MDRI:actin ratio of the samples ranges from 0.00008–0.34 (Table 1), with the MDRI:actin ratio of the highly resistant cell line being 0.17 and of the...
weakly resistant cell line being 0.0024. The numbers are small, which is due to the abundant expression of the internal standard gene, β-actin; thus, it is important to evaluate relative and not absolute numbers.

**MDRI Expression in Normal Endometrium.** Benign endometrium from 38 patients was collected, including 15 patients with proliferative endometrium (average age, 46 ± 6 years), 8 patients with secretory endometrium (average age, 44 ± 4 years), and 15 patients with atrophic endometrium (average age, 66 ± 12 years). The histology of each sample was examined by a pathologist (J. J.) to confirm the presence of endometrial tissue. Fig. 3A is a plot of the 95% confidence interval of the MDRI levels for the three groups of patients. The MDRI levels in the atrophic endometrium were significantly higher than those in the proliferative endometrium group (P = 0.0024). There were insufficient numbers of patients to evaluate the MDRI levels of early and late proliferative endometrium as compared to early and late secretory endometrium.

**MDRI Expression in Endometrial Carcinoma.** A total of 43 endometrial carcinoma tumor samples were included in the study. The samples were divided into 3 groups; 18 specimens were well-differentiated adenocarcinoma, 11 were moderately or poorly differentiated adenocarcinomas, and 14 specimens were from tumors of poor histology, including papillary serous, clear cell, and adenosquamous cell tumors. Each sample was examined by a pathologist (J. J.). The distribution of MDRI expression in these three groups and in the control (benign postmenopausal endometrium) is shown in Fig. 3B. MDRI expression in the benign postmenopausal endometrium was significantly higher than any of the tumor groups (P = 0.0001). When MDRI expression in these tumor samples was categorized based on various tumor prognostic
Fig. 2 Autoradiography of MDR1 and β-actin transcription products and subsequent quantitative analysis for a tumor sample (A), a low resistant cell line (B), and a high resistant cell line (C). Insert, the linear region of the curve. The slope (in cpm/µl) and the correlation coefficient are given for each linear plot.
Table 1  Correlation between immunocytochemistry and PCR analysis of MDR1 expression

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a Em, endometrium; well-diff adenoca, well-differentiated adenocarcinoma; mod diff adenoca, moderately differentiated adenocarcinoma; poorly diff adenoca, poorly differentiated adenocarcinoma; pap serous, papillary serous carcinoma; ca, carcinoma.

b PCR values are converted to a 10-point scoring system. The range of each score is as follows: 1, \(0.000-0.00025\); 2, \(0.00025-0.0005\); 3, \(0.0005-0.00075\); 4, \(0.00075-0.001\); 5, \(0.001-0.0025\); 6, \(0.0025-0.0050\); 7, \(0.005-0.0075\); 8, \(0.0075-0.010\); 9, \(0.010-0.025\); and 10, \(0.025-0.050\).

c Determined by microscopic examination. Four categories were defined: <25, 25–50, 50–75, and 75–100%.

d Discrepancies exist between the PCR values and immunohistochemistry for this sample.

The association between tumor type and nuclear grade with hormone receptor levels was examined (data not shown). Hormone receptor levels correlate with the degree of differentiation and nuclear grade, as reported previously (39–42). The level of MDR1 expression seems highest in the most-differentiated tumors as indicated by nuclear grade. However, although both hormone receptors and MDR1 are at their highest levels in well-differentiated tumors, there is no statistically significant relationship between the presence of receptors and MDR1 expression.

**Immunohistochemistry.** Quantitative RT-PCR allowed detection of MDR1 RNA levels. However, to confirm the presence of the product of the MDR1 gene (P-gp), immunohistochemistry was performed. It is important to note that positive P-gp staining with MM4.17 is observed in all of the sample slides. Fig. 4 illustrates some of the staining of both the control...
and tissue samples. Fig. 4A is the staining of SKVLB cells prepared and fixed in acetone at 0°C, demonstrating strong membranous staining and moderate to strong perinuclear staining. As a negative control, a moderately differentiated adenocarcinoma sample was stained with mouse IgG in Fig. 4B, and the same tissue was stained with the MM4.17 monoclonal antibody in Fig. 4C, showing weak to moderate nonhomogeneous cytoplasmic staining (including the perinuclear region) plus a few discrete areas of membranous staining. Fig. 4C scored +, which corresponded to its RT-PCR score of 1 (see the next section for RT-PCR scoring system). Fig. 4D showed a poorly differentiated adenocarcinoma tissue demonstrating weak nonhomogeneous cytoplasmic and perinuclear staining, with a score of + from immunohistochemistry and a 4 from RT-PCR. Fig. 4E and 4F were slides of two separate well-differentiated adenocarcinoma tissues indicating moderate to strong luminal staining with moderate nonhomogenous diffuse cytoplasmic staining. The immunohistochemistry scores were ++ for both samples, whereas the RT-PCR scores were 5 and 6, respectively. Fig. 4G was a postmenopausal cystic atrophic endometrium showing strong luminal staining with moderate diffuse cytoplasmic staining and a score of ++ for immunohistochemistry and a 5 for RT-PCR. Fig. 4H was a tissue from a patient with a history of both breast and colon cancer who had a poorly differentiated endometrial adenocarcinoma. The staining in this tissue was diffusely intense. A score of +++ correlated with a quantitative RT-PCR value of 10, which was the highest among all samples.

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1988 MDR1 Expression in Human Endometrium

Correlation between Immunohistochemistry and PCR Analysis of MDR1 Expression. The RT-PCR values were simplified to a 10-point scoring system to correlate with the immunohistochemistry values. In this scoring system, a 1–4 score corresponds to a + in immunohistochemistry, a 5–8 score corresponds to a ++ in immunohistochemistry, and a 9–10 score corresponds to a +++ in immunohistochemistry. A total of 45 samples were stained, and the pathologist (H.-J.S.) scored the staining without prior knowledge of the PCR results (Table 1). An 80% correlation (36 of 45 samples) was observed between PCR and immunohistochemistry staining.

The percentage of endometrial tissue in each sample slide was determined by a single pathologist (J.-J.), and the numbers are given in Table 1. The majority of the malignant samples had a high endometrial component, whereas many benign samples had a higher stromal component, which could result in an underestimation of the actual MDR1 levels by at least 2-fold.

DISCUSSION

MDR is an important and well-documented mechanism of drug resistance. Many studies have clearly demonstrated that the level of MDR1 mRNA correlates with the degree of resistance (25, 28). Resistant tumors are divided into two categories, intrinsic and acquired. Tumors derived from tissues that normally express high levels of MDR1 are generally intrinsically refractory to chemotherapeutic agents of natural product origin (20, 22). Acquired resistance is the term given to tumors that have high levels of MDR1 only after treatment (19, 21, 23).

From many studies performed in rodents, it is clear that the mdr1b gene is expressed in the uterus and is regulated by progesterone (29, 30, 43). Because human and mouse mdr genes share a high degree of homology in their structure, function, and tissue distribution (15, 16, 44–46), it is reasonable that MDR1 is intrinsically expressed in the human uterus. A preliminary retrospective study by Axiots et al. (34) using immunohistochemistry suggested that MDR1 was present in the uterus and that its expression may be regulated by progesterone. This has interesting clinical implications. If MDR1 expression is regulated by progesterone, then therapy with the progesterone receptor antagonist (RU486) may prevent the onset of resistance in endometrial carcinoma. We therefore sought to determine the levels of MDR1 expression in endometrial carcinoma.

We chose to use a modification of RT-PCR methodology to increase the sensitivity of RNA detection in samples of benign and malignant endometrium. The level of MDR1 was compared to an internal standard (38), and a relative value was calculated. Two independent means to determine the MDR1 levels were used because each tissue block was divided in half, one section for PCR and the other for immunohistochemistry. Brophy et al. (47) recommended that RT-PCR be used as the standard technique to measure MDR1 levels for assessing clinical specimens because of its relative simplicity and specificity and suggested that an additional technique, such as immunohistochemistry, would be useful to detect heterogeneity of P-gp expression.

We used as controls benign tissue obtained from patients undergoing hysterectomy. Based on histological criteria, they were classified as premenopausal or postmenopausal. Our data revealed significantly higher levels of MDR1 expressed in the postmenopausal as compared to the premenopausal uterus. From the immunohistochemistry, it is clear that P-gp is expressed primarily in the epithelial cell layer in both pre- and postmenopausal women. By separating premenopausal samples into proliferative and secretory groups, no statistically significant difference in MDR1 level was observed, although a trend was noted with more expression in the secretory phase. A larger sample size may yield a statistical significance between the two groups.

A previous report on MDR1 expression in three postmenopausal endometrial specimens using immunohistochemistry with monoclonal antibody C219 did not detect any staining (32). This difference from our study may be partly due to the sensitivity of the antibody used. It is not clear why the levels of MDR1 are higher in postmenopausal women, and this requires further investigation.

Forty-three tumors derived from uterine tissue were analyzed. The level of MDR1 gene expression was higher in the control postmenopausal group than in the tumor groups and decreased markedly with an increase in grade. A decrease in MDR1 expression has also been reported in renal cell carcinoma
Fig. 4 The immunohistochemical staining of P-gp by monoclonal antibody MM4.17. A, SKVLB cell staining, a positive control (×400). B, a moderately differentiated adenocarcinoma stained with mouse IgG instead of MM4.17 as a negative control (×400). C, the same paraffin section as B stained with MM4.17, showing weak to moderately nonhomogeneous intracellular cytoplasmic staining (including the perinuclear region) plus a few discrete membranous staining areas (×400). Immunoscore, +; PCR score, 1. D, poorly differentiated adenocarcinoma showing weak nonhomogenous diffuse cytoplasmic and perinuclear staining. Immunoscore, +; PCR score, 4 (×400). E, well-differentiated adenocarcinoma showing moderate to strong continuous and discrete luminal staining with moderate nonhomogenous diffuse cytoplasmic staining. Immunoscore, +++; PCR score, 5 (×400). F, another well-differentiated adenocarcinoma. Immunoscore, +++; PCR score, 6 (×400). G, postmenopausal endometrium with cystic atrophy showing moderate to strong luminal staining with moderate diffuse cytoplasmic staining. Immunoscore, +++; PCR score, 5 (×250). H, poorly differentiated adenocarcinoma showing strong membranous and cytoplasmic staining. Immunoscore, +++; PCR score, 10 (×400).
(48), in which MDR1 levels were high in normal renal tissue but low in malignant tissue. It is possible that P-gp has a specific role in the normal differentiated tissue and as the tumors become less differentiated and more virulent, expression of P-gp is lost. Clinically, it is known that patients with high-grade tumors usually have an initial response to chemotherapy, which is consistent with their low MDR1 level. However, these responses are usually of short duration, which might suggest an up-regulation of MDR1 expression. It would be interesting to test this hypothesis by collecting endometrial tissue before and after chemotherapy.

Except for two patients, there was good correlation between MDR1 expression and the grade and histology of tumor. As noted in Fig. 3B, the patients with the highest MDR1 levels (0.034 and 0.021) were in the moderate/poorly differentiated adenocarcinoma group. The patient with a tissue MDR1 level of 0.034 had a poorly differentiated endometrial adenocarcinoma with serosal involvement and lymphovascular invasion. Her history was also significant for breast and colon cancer, for which she was treated surgically. Tamoxifen was given for breast cancer, and chemotherapy was given for colon cancer. The high level of MDR1 is probably a result of chemotherapeutic treatment leading to induction of MDR1. The other patient had a superficially invasive poorly differentiated adenocarcinoma and was not treated further after surgery. If these two patients were excluded from the statistical analysis because the MDR1 expression was greater than 2 SDs from the mean, then the level of MDR1 expression in well-differentiated adenocarcinoma would be significantly different from that of the other tumor types.

The levels of MDR1 observed in all of the tumor samples (highest number, 0.034) were not as high as that observed in the highly drug-resistant culture cell line (MDR1 level = 0.17; see Fig. 2). The level of resistance seen in many cancer cells is only 3–10-fold, corresponding to a small increase in mRNA. Although the increase in mRNA is modest, it is sufficient to confer clinically significant resistance.

We have also observed some heterogeneity in MDR1 gene expression within a tumor type. P-gp is expressed only in the single cell layer that makes up the endometrium. No expression is detected in the stroma. Because some samples may have a higher percentage of stromal cells than others, the MDR1 levels calculated by RT-PCR may be artificially lowered by dilution. MDR1 levels seen in the benign postmenopausal endometrium are higher than in malignant tissue. The presence of a greater than 50% stromal component in the benign endometrium may underestimate the actual MDR1 levels in the benign postmenopausal endometrium.

This is the first study using the MM4.17 monoclonal antibody to detect MDR1 in endometrial tissue. MM4.17 was selected for these experiments for several reasons: (a) it is the only antibody that recognizes a continuous linear epitope of eight amino acids identified in the P-gp extracellular domain (49). Most of the other P-gp antibodies bind to epitopes in the cytoplasmic domains. Although MRK16 binds to an extracellular epitope, it is conformationally sensitive, requiring the presence of two discontinuous sequences; (b) to assess the feasibility of using paraffin sections with the MM4.17 antibody, SKVLB cells were fixed in 3.7% formalin and permeabilized by methanol or ethanol, a process that mimicked the preparation of paraffin sections (data not shown). The SKVLB cells were then stained with MM4.17 antibody, which yielded a positive result. However, staining of 8226 Dox40, a doxorubicin-resistant multiple myeloma cell line, by MRK 16 antibody yielded a negative result as reported by Grogan et al. (50) using the same slide preparation, indicating the sensitivity of MM4.17 over MRK16; and (c) a comparison of staining between MM4.17 and C219 in paraffin sections of selected samples revealed that MM4.17 was more sensitive than C219 antibody (data not shown). However, because the epitope of MM4.17 is eight amino acids, there is an increased chance of cross-reactivity with other cellular elements. Positive staining has been seen occasionally in stromal cells, macrophages, or endothelial cells in our samples.

Several other groups have used immunohistochemistry to measure MDR1 levels in the endometrium and endometrial adenocarcinoma (31–33, 51), but none have demonstrated the sensitivity achieved in our study using MM4.17. Axiotis et al. (34) used murine monoclonal antibodies C219, C494, and JSB-1 to stain 36 endometrial tumor samples from formalin-fixed paraffin-embedded sections. The samples were not consistently stained by all three antibodies. Positive staining was noted in 72% of the tumors with at least one of the monoclonal antibodies. The percentage of positive staining with each antibody ranged from 50–67%. Many of the samples only showed Golgi and perinuclear staining. Recently, Kodama et al. (32) examined human endometrium by immunohistochemistry using C219. They were able to show that the P-gp expression was inversely correlated with tumor differentiation, which is consistent with our results. They did not, however, detect any expression in postmenopausal endometrium. Because there were only three postmenopausal patients, their data should be verified with a larger sample. All of our postmenopausal samples showed positive staining that was confirmed by quantitative RT-PCR.

In summary, we have used two methods to measure the levels of MDR1 expression and P-gp production in benign and malignant endometrium. We have described results with an antibody to an external epitope of human P-gp that may be more sensitive for staining paraffin sections than those previously available. MDR1 expression is highest in postmenopausal endometrium, being localized specifically to the epithelial layer of the endometrium. Our data suggest that MDR1 expression is not a major component of the drug resistance observed in primary endometrial tumors.

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Analysis of MDR1 expression in normal and malignant endometrium by reverse transcription-polymerase chain reaction and immunohistochemistry.

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