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P-glycoproteins encoded by *mdr1b* in murine gravid uterus and multidrug resistant tumor cell lines are differentially glycosylated

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There are 3 members of the multidrug-resitance gene family expressed in mouse. Only one of these, *mdr1b*, and its gene product P-glycoprotein are induced to high levels in the mouse endometrium during pregnancy. It is shown here that P-glycoprotein in the gravid uterus is significantly larger (M_r 155000) compared to P-glycoprotein encoded by *mdr1b* in a murine multidrug-resistant cell line (M_r 140000). However, both species co-migrate after enzymatic removal of *N*-linked sugars (M_r 125000). These results demonstrate that differential glycosylation of the *mdr1b* gene product contributes to molecular heterogeneity found in P-glycoprotein from normal and multidrug-resistant cells.

Multidrug resistance; P-glycoprotein; Glycosylation; Pregnancy; Chemotherapy; Mouse endometrium

1. INTRODUCTION

When mammalian tumor cells are selected in culture for resistance to a single antitumor agent of natural product origin, they will often display cross-resistance to a wide variety of structurally and functionally dissimilar chemotherapeutic agents. This type of multidrug resistance is due to the increased expression of specific members of a multigene family, termed *mdr*. The *mdr* genes encode a membrane glycoprotein, designated P-glycoprotein, which functions as an energy-dependent drug efflux pump with broad specificity (see [1]).

In mouse, 3 genes comprising this multigene family have been identified [2–5] and have been designated *mdr1* [2], *mdr2* [3] and *mdr3* [4]. *mdr1* and *mdr3*, also known as *mdr1b* and *mdr1a* [5], respectively, have been associated with the multidrug-resistance phenotype [2,5]. *mdr1* has been shown to confer multidrug resistance by transfection of its cDNA into drug-sensitive cells [2]. Furthermore, independently isolated mouse multidrug-resistant cell lines that make P-glycoprotein encoded by either *mdr1a* or *mdr1b* have been isolated [5]. Mouse *mdr2* is a highly homologous gene but does not appear to be capable of conveying multidrug resistance [3]. In this report we will make use of the designations *mdr1a* and *mdr1b*.

mdr1a and *mdr1b* encode 120- and 125-kDa P-glycoprotein precursors, respectively [5,6]. These precursors contain approx. 5 kDa of high mannose type oligosaccharides which are added co-translationally. The 120- and 125-kDa precursors mature to their final molecular masses of approx. 130 and 140 kDa, respectively, when the oligosaccharides are processed to complex-type sugar [6,7]. The mature protein is also phosphorylated [8–11].

mdr gene expression is dramatically increased in the gravid murine uterus [12]. The increased expression of P-glycoprotein is specifically localized to the luminal surface of the secretory epithelial cells within the endometrium. By using Northern blot analysis with gene specific probes, it has been demonstrated that the gravid endometrium expresses only *mdr1b* mRNA (referred to as *mdr1* in [4]). These results suggest that the *mdr1b* gene product plays an important role during normal gestation. The endogenous substrate(s) for P-glycoprotein have not been identified. However, progesterone may play a role in transport since it can interact with P-glycoprotein and reverse multidrug resistance [13].

Although differential glycosylation of P-glycoprotein occurs in multidrug-resistant cell lines [6,7], little is known about P-glycoprotein gene products in vivo. To this end, we compared P-glycoprotein from the uterus to that found in multidrug-resistant cell lines.

2. MATERIALS AND METHODS

2.1. *Cell lines*

Two J774.2 mouse macrophage-like cells, designated J7.V1-1 and J7.V3-1, were selected for resistance to vinblastine (VBL) and maintained in drug [7]. J7.V1-1 and J7.V3-1 denote distinct, independently isolated cell lines that are maintained in 1.0 μM drug.

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2.2. *Endometrial sample preparation*

C57Bl/6J mice were mated overnight and separated the next morning which was considered to be gestation day 1. On day 15, mice were sacrificed and the uterus separated from the placenta and embryo. Microsomal membranes from the endometrium and plasma-membrane enriched subcellular fractions from multidrug-resistant cells were prepared as described [7,13].

2.3. *Membrane preparation and Western blot analysis*

Membrane protein was solubilized in sample buffer (without heating; see [14]), resolved by polyacrylamide gel electrophoresis [15] and transferred to nitrocellulose [16] or electroeluted. Blots were probed as previously described with a polyclonal antibody, R3, specific for P-glycoprotein [7,17]. Electroeluted material was incubated with peptide N-glycosidase F (PNGaseF) for 16 h at 37°C [6], re-electrophoresed and transferred to nitrocellulose.

3. RESULTS AND DISCUSSION

P-glycoprotein from the gravid uterus and multidrug-resistant tumor cells were compared using the R3 antiserum. Fig.1 (left panel) demonstrates that P-glycoprotein from gravid uterus migrated with an M_r of 155000 whereas the P-glycoprotein from the multidrug-resistant murine cell line, J7.V1-1, migrated with an M_r of 140000. No immunoreactive P-glycoprotein was observed with pre-immune serum (fig.1, right panel). In addition, P-glycoprotein was not detected in the nonpregnant uterus, which is consistent with the very low levels of *mdr* mRNA detected ([12] and data not shown). Since it is known that P-glycoprotein in the endometrium and J7.V1-1 cells is encoded by *mdr1b* [4,5], these results suggested that compared to tumor cells in culture, the gravid uterus P-glycoprotein was differentially modified.

To evaluate if post-translational *N*-linked glycosylation was responsible for the differences in molecular weight that were observed, the deglycosylated species were compared. P-glycoprotein from the gravid uterus was compared with two multidrug-resistant cell lines, J7.V1-1 and J7.V3-1, which make P-glycoprotein encoded by the *mdr1b* and *mdr1a* genes, respectively [5]. The mature *mdr1b* gene product migrates at approx. 140 kDa, while the *mdr1a* gene product migrates at 130 kDa. When enzymatically deglycosylated, the *mdr1b* and *mdr1a* gene products migrate at 125 and 120 kDa, respectively ([6,7] and fig.2). To make a comparison between P-glycoprotein from the endometrium and multidrug-resistant cell lines, P-glycoprotein that had been resolved in gels was electroeluted and digested with peptide N-glycosidase F. This indirect procedure was necessary, since the protein degraded when total membrane preparations were digested directly. The deglycosylated protein was then resolved in gels and transferred to nitrocellulose in preparation for immunoblot analysis. Fig.2 demonstrates that the deglycosylated form of P-glycoprotein from the gravid uterus co-migrated with the 125-kDa deglycosylated species observed in the J7.V1-1 cell line and not with the 120-kDa species found in the J7.V3-1 cell line.

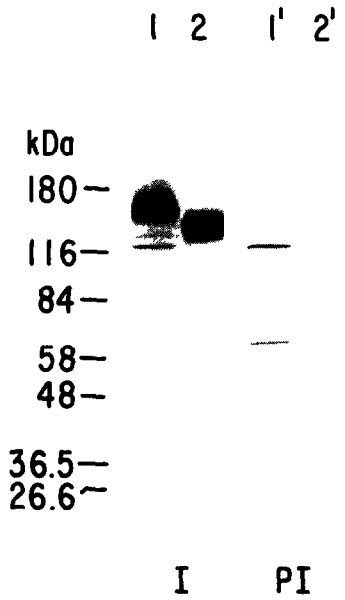

Fig.1. Western blot analysis of P-glycoprotein from gravid uterus and J7.V1-1 multidrug-resistant cells. Left panel shows anti-P-glycoprotein antiserum (I) binding to membrane preparations from (1) gravid uterus and (2) J7.V1-1 cells. Right panel shows pre-immune serum (PI) binding to membrane preparations from (1') gravid uterus and (2') J7.V1-1 cells. Molecular mass marker positions are given on the left.

Therefore, P-glycoprotein from the gravid uterus migrated at an apparent larger molecular weight because of differential *N*-linked glycosylation.

It is most likely that differential glycosylation between P-glycoproteins from the endometrium and J7.V1-1 cells is due to unique processing of oligosaccharides on an identical amino acid backbone, since gene specific probes have been used to show that endometrial cells and J7.V1-1 cells encode P-glycoproteins derived from *mdr1b* [4,5]. The latter conclusion has been confirmed with an antibody that is specific for the *mdr1b* gene product since this antibody recognizes endometrial P-glycoprotein (Hsu and Horwitz, personal observation). A less likely alternative in-

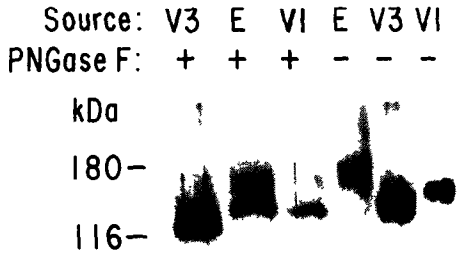

Fig.2. Western blot analysis of deglycosylated and native P-glycoprotein. The membrane preparations were treated with PNGase F (+) or enzyme buffer (−) as indicated. V3 and V1 indicate membrane preparations from multidrug-resistant cell lines J7.V3-1 and J7.V1-1, respectively. E signifies a membrane preparation from gestation day 15 uterus. Molecular mass markers are given on the left.

terpretation is that both P-glycoproteins are encoded by *mdr1b* homologs in which there is diversity in the coding sequence in the region of *N*-linked glycosylation. Alternate splicing, which has been observed in a homologous *mdr* gene found in human liver [18], could create such diversity.

Differential *N*-linked glycosylation of the *mdr1b* gene product can increase the apparent molecular mass of the 125 kDa-precursor by 10–17 kDa in independently isolated J774.2-derived multidrug-resistant cell lines [6,7]. It can also increase the mobility of the P-glycoprotein precursor expressed in hamster multidrug-resistant cells by 30 kDa [14]. It remains unclear if differences in glycosylation alter the capacity of P-glycoprotein to transport endogenous compounds or natural products. Some data suggest that such differences may not generally influence the function of P-glycoprotein since lectin mutant cell lines can become multidrug resistant [19] and multidrug-resistant cells treated with tunicamycin remain resistant to vinblastine [20]. However, the differences in carbohydrate structure indicate that the extracellular portion of P-glycoprotein in normal and multidrug-resistant cell lines can be dissimilar.

To our knowledge, this is the first example of differential modification of P-glycoprotein from a normal tissue compared to that of a multidrug-resistant cell line. Further biochemical analysis and in vitro systems with gravid uterine epithelial cells will be required to evaluate further the significance of such diversity.

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