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PERSPECTIVE

Progesterone Acts via Progesterone Receptors A and B to Regulate Breast Cancer Resistance Protein Expression

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ABSTRACT

The breast cancer resistance protein (BCRP; ABCG2) is an ATP-dependent efflux multidrug transporter that belongs to the G family of half-transporters that consist of six transmembrane-spanning domains and must homodimerize to form the active membrane transporter. It is expressed in the apical plasma membrane domain of the small intestine, endothelium, and liver, where it has been shown to play an important role in limiting drug absorption and distribution and in enhancing drug clearance, respectively. BCRP is also expressed in the apical membrane of mammary alveolar epithelia, where it mediates efflux of substrates into milk, and in the placental syncytiotrophoblasts, where it reduces fetal exposure to these substrates. BCRP substrates include numerous drugs (topotecan, nitrofurantoin, cimetidine) as well as food carcinogens (2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine) and the vitamins riboflavin and folic acid. BCRP expression is regulated by a number of nuclear transcription factors, including the peroxisome proliferator-activated receptor- γ and Hif-1. This issue of *Molecular Pharmacology* includes a study (p. 845) now conclusively demonstrating that progesterone acts via the progesterone A and B receptors to regulate BCRP expression in a placental cell line.

In this issue of *Molecular Pharmacology*, Wang et al. (2008) provide data clearly demonstrating the ability of progesterone to increase expression of breast cancer resistance protein (BCRP; ABCG2) in BeWo cells via a progesterone response element in the *BCRP* promoter. BCRP is an ATP-dependent efflux transporter and belongs to the G family of half-transporters that consists of six transmembrane spanning domains and homodimerizes to form the active membrane transporter. BCRP was initially cloned from a breast cancer cell line that was highly doxorubicin-resistant (Doyle et al., 1998), and thus named the breast cancer resistance protein gene. The gene was also cloned by two other groups, and named *MXR* (mitoxantrone resistance) and *ABCP* [based on its identity as an ATP-binding cassette (ABC) protein expressed in placenta] (Allikmets et al., 1998; Miyake et al., 1999). Not surprisingly, BCRP expression in cell lines was shown to confer resistance to several cancer chemotherapeutic drugs, including mitoxantrone, doxorubicin, daunorubicin, and topotecan. However, BCRP shows a broad substrate specificity, and its substrates include xenobiotics such as dietary carcinogens and toxic metabolic products (Jonker et al., 2002; van Herwaarden et al., 2003), as well as antibiotics (nitrofurantoin) and antihistamines (cimetidine), among others. With some exceptions, few endogenous substrates have been recognized for most of the 50 known mammalian ABC transporters, but several endogenous molecules with important physiological functions are known to be BCRP substrates, including riboflavin, folic acid, and sulfated conjugates of steroid hormones (van Herwaarden and Schinkel, 2006).

From a functional perspective, BCRP seems to play a protective role for the host and its fetus. BCRP expression on the apical surface of endothelium, the small intestinal epithelium, the liver canalicular membrane, and placental syncytiotrophoblasts (Maliepaard et al., 2001) is consistent with the prototypical role of ABC transporters in modulating drug disposition by limiting absorption and drug distribution and by enhancing drug clearance. In contrast, several recent pub-

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ABBREVIATIONS: BCRP, breast cancer resistance protein; ABC, ATP-binding cassette; RU-486, mifepristone; PR, progesterone receptor; ICI 182,780, fulvestrant.

lications, following a seminal article by Jonker et al. (2005), have demonstrated markedly increased excretion of BCRP substrates in milk during lactation (e.g., riboflavin, cimetidine, nitrofurantoin, topotecan). This is consistent with the expression of BCRP in the apical domain of mammary epithelium, and although it demonstrates its role as an important transporter for endogenous substrates (e.g., riboflavin), it also highlights the risk for drug exposure to nursing neonates if their mothers are being treated with drugs that are BCRP substrates (Oo et al., 1995; Gerk et al., 2001).

Several groups have addressed the question of hormonal regulation of BCRP expression in pregnancy and in the placenta specifically. BCRP expression in human placenta at preterm (~28 weeks) is approximately twice that at term (39 weeks) (Meyer zu Schwabedissen et al., 2006), a pattern similar to that in the mouse (Wang et al., 2006) and rat (Yasuda et al., 2005). Estradiol and progesterone have been shown to regulate BCRP expression in cancer cell lines, including BeWo cells, a cell line derived from human placental choriocarcinoma (Ee et al., 2004; Imai et al., 2005; Wang et al., 2006). In the current study, Wang et al. (2008) transfected BeWo cells with a −1285/+362 BCRP promoter luciferase construct and treated them with progesterone in the presence of aminoglutethimide to inhibit endogenous progesterone production. Under these conditions, concentrations of progesterone known to activate the progesterone receptor (i.e., 10^{-7} M) were highly effective in increasing BCRP expression. RU-486, the progesterone receptor antagonist, significantly decreased basal luciferase activity, thus implicating the classic progesterone receptors (PR) in mediating this effect. Both the A and B forms of PR were identified in BeWo cells; PRB expression was predominant. Transfection of PRB dramatically (9-fold) increased BCRP expression in response to progesterone, whereas transfection of PRA either had little effect or decreased BCRP expression. Deletion and mutation analyses identified a functional progesterone response element at −187 to −173 in the BCRP promoter that responded to progesterone in the presence of PRB and was effectively antagonized by RU-486. Together, these data demonstrate that progesterone can act via PRB to increase expression of BCRP and provide an explanation for earlier findings (Wang et al., 2006) that only high concentrations (10^{-5} M) of progesterone induced BCRP expression in BeWo cells, an effect that was not antagonized by RU-486. The present study supports the hypothesis that, because of the endogenous production of progesterone by BeWo cells, the classic PR was already near saturation levels, so that concentrations known to activate classic PR had little or no effect.

A number of important questions remain, however. As noted by Wang et al. (2008), the progesterone response element identified here is the same element identified previously by Ee et al. (2004) as an estrogen response element (Fig. 1). This response element binds to both the PR and estrogen receptor, as determined by electrophoretic mobility shift assays and responses to 10 nM estradiol are antagonized by 0.1 to 1 μM ICI 182,780. However, whereas Ee et al. (2004) showed that estrogens increase BCRP mRNA expression, others (Imai et al., 2005; Wang et al., 2006) showed that estrogens post-transcriptionally decrease BCRP protein expression. A further complexity arises because estradiol can induce expression of PRB (Flötotto et al., 2004; Wang et al., 2006), thus presumably potentiating the up-regulation by progesterone, a mechanism proposed by Wang et al. (2008) in the current studies.

Consensus PRE	5'GGTACANNNTGTTCT3'
Palindrome PRE	5'AGAACANNNTGTTCT3'
BCRP PRE/ERE	5'ACGGCAGGGTGACCC3'
Consensus ERE	5'PuGGTCANNNTGACCPy 3'

Fig. 1. Sequence of the consensus progesterone and estrogen response elements (PRE, ERE, respectively) detected in the BCRP promoter at −187 to −173. The underlined bases represent those identical to either of the reference PREs.

Although the current work by Wang et al. (2008) demonstrates the mechanism via which progesterone regulates BCRP expression in BeWo cells, additional effectors have been identified as important for the regulation of BCRP expression, but in different cellular contexts. First, Krishnamurthy et al. (2004) demonstrated that BCRP expression is critical for protecting hematopoietic stem cells and facilitates cell survival under hypoxic stress conditions. This seems to be regulated via a Hif-1-mediated pathway and is perhaps important for cancer cell survival in poorly perfused tumor regions. In relation to the current work, the Hif-1 pathway is also important for placental vascularization and trophoblast differentiation (Adelman et al., 2000; Cowden Dahl et al., 2005), but its role in BCRP expression in trophoblast cells has not been demonstrated. Second, the peroxisome proliferator-activated receptor-γ was shown to increase ABCG2 expression in dendritic cells after heterodimerization with the nuclear receptor retinoid X receptor and direct binding to DNA upstream of the ABCG2 promoter region, acting as transcriptional enhancer elements (Szatmari et al., 2006). Although this observation was in a different cellular context, peroxisome proliferator-activated receptor-γ and retinoid X receptor are both expressed in human trophoblasts and are known to modulate cellular differentiation (Schaiff et al., 2000). The relative importance of these two pathways in placental BCRP expression has not yet been investigated. Recent studies have shown decreased expression of placental BCRP in idiopathic human fetal growth restriction (Evseenko et al., 2007), and women with fetal growth restriction had lower estradiol and progesterone levels after week 34 compared with women with normal pregnancies or preeclampsia (Salas et al., 2006). Although Evseenko et al. (2007) postulated that BCRP is functioning as a placental survival factor, an endogenous substrate was not identified. It could be important to determine whether the regulation of BCRP by progesterone and estradiol have a mechanistic tie to idiopathic fetal growth restriction or other regulators of plasma volume in pregnancy.

The interactions between estradiol, progesterone, and their respective receptors are complex but probably contribute to the changes in expression of Bcrp in mouse tissues in pregnancy (Wang et al., 2006). Thus, renal, hepatic and placental Bcrp expression are maximal at midgestation (days 10–15); expression in kidney and liver are increased 3-fold relative to nonpregnant female controls. The functional importance of the increases in liver BCRP expression during pregnancy remains to be elucidated, as are the mechanism(s) that influences these changes. Whether similar changes occur in human liver in pregnancy is not known. Expression of Mrp2 in rat liver is decreased approximately 50% in pregnancy (Cao et al., 2002), so that increased Bcrp expression might compensate and provide a means of hepatic clearance for those compounds that are substrates for both Mrp2 and Bcrp. In summary, the present work provides new insights into the mechanisms of regulation of

BCRP that will enable additional studies for understanding the role of BCRP in drug clearance, and perhaps in placental development, and also points to the need for more studies to investigate the clinical implications of these intriguing findings.

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