P-glycoprotein and breast cancer resistance protein expression in human placentae of various gestational ages

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Mathias, Anita A., Jane Hitti, and Jashvant D. Unadkat. P-glycoprotein and breast cancer resistance protein expression in human placentae of various gestational ages. Am J Physiol Regul Integr Comp Physiol 289: R963–R969, 2005.—Placental efflux transporters such as P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP) protect the developing fetus from exposure to potentially toxic xenobiotics. However, little is known about the expression of these transporters in human placentae of different gestational ages. Therefore, we quantified the expression of P-gp and BCRP in human placentae of different gestational ages. We also measured the expression of various nuclear regulatory factors such as the pregnane X receptor to determine whether their expression also changes with gestational age. Syncitial microvillous plasma membranes were isolated from human placentae of various gestational ages (60–90 days, 90–120 days, and full-term C-section placentae). P-gp and BCRP expression were measured in these preparations by Western blot analysis followed by an ELISA. Expression (mRNA) of P-gp, BCRP, nuclear regulatory factors (mRNA and protein) in the placentae were quantified by quantitative real-timePCR. P-gp expression (relative to that of alkaline phosphatase) was significantly (P < 0.05) higher (44.8-fold as protein; 6.5-fold as mRNA) in early gestational age human placentae (60–90 days) vs. term placentae. In contrast, BCRP expression (protein and mRNA) and nuclear regulatory factors (mRNA) expression in placentae tissue did not change significantly with gestational age. However, placentae expression of P-gp and human chorionic gonadotropin-β (hCG-β) transcripts was highly correlated (r = 0.73; P < 0.0001; Spearman rank correlation). Expression of P-gp, but not BCRP, decreases dramatically with gestational age in human placentae. This decrease in P-gp expression is not caused by a change in expression of nuclear receptor transcripts but appears to be related to hCG-β expression. The placental P-gp expression appears to be upregulated in early pregnancy to protect the fetus from xenobiotic toxicity at a time when it is most vulnerable to such toxicity.

besides facilitating the exchange of nutrients, the placenta serves as a protective barrier that modulates the exposure of the fetus to xenobiotics, including drugs (42). One mechanism by which the placenta functions as a protective barrier is by the expression of efflux transporters on the apical membrane of the syncytiotrophoblasts, the cells separating maternal and fetal circulations. Of the efflux transporters expressed there, the most abundant are P-glycoprotein (P-gp), MDR1, or ABCB1 gene product and the breast cancer resistance protein (BCRP/ABCG2) gene product (1, 6, 38).

P-gp and BCRP are multidrug-resistant proteins that belong to the adenosine triphosphate-binding cassette family of transporters. However, unlike P-gp, BCRP is a half transporter and is evolutionarily distinct from P-gp (7, 30). The localization of P-gp and BCRP on the apical membrane of the syncytiotrophoblasts suggests that these transporters are important in preventing entry of potential toxins into the fetal compartment. Lankas et al. (17) have demonstrated that deficiency in the mouse placental P-gp enhances fetal susceptibility to chemically induced birth defects by avermectins. Smit et al. (34) demonstrated that the accumulation of the P-gp substrates digoxin and saquinavir in mdrla/1b null fetuses was 2.4-fold and 5–7-fold greater than that in wild-type fetuses. Furthermore, this difference in accumulation was abrogated in wild-type mice by the presence of a P-gp and BCRP inhibitor, GG918. Likewise, Jonker et al. (12) have demonstrated a protective role of placental BCRP. They found that the fetal distribution of topotecan (a BCRP substrate) was 2.0-fold greater in pregnant, GG918-treated P-gp-deficient mice than in pregnant, vehicle-treated mice. Collectively, these data suggest that placental P-gp and BCRP play a crucial role in preventing drugs from entering the fetal compartment.

Although placental P-gp and BCRP are known to be important modulators of fetal drug exposure, little is known about the expression of these transporters in human placentae of different gestational ages. This is an important question to address because the fetus is most vulnerable to xenobiotic toxicity early in pregnancy during the organogenesis period. By immunohistochemistry, MacFarland et al. (23) examined first-trimester and term human placentae and observed that, in first-trimester human placentae, P-gp was predominantly located on the syncytiotrophoblast microvillus border and that its expression decreased in the later stages of pregnancy. In addition, in later stages of pregnancy, P-gp expression was not limited to the trophoblasts. These data suggest that P-gp may have distinct functions during the different stages of placental development, and its expression may be under developmental control. However, this immunohistochemistry study was qualitative and not quantitative. In this manuscript, we report a quantitative study of the expression (protein and mRNA) of P-gp and BCRP in human placentae of different gestational ages. Because we

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observed a gestational age-dependent change in placental P-gp expression, we explored potential mechanisms by which this expression may be regulated. To do so, we determined the placental expression of various regulatory factors such as pregnane X receptor (PXR; pregnane or steroid xenobiobic receptor), constitutive androstane receptor (CAR), vitamin D receptor (VDR) and hepatocyte nuclear factor-4α (HNF-4α) that have previously been reported to modulate expression of intestinal and hepatic P-gp or cytochrome P-450

MATERIALS AND METHODS

Reagents. Primary antibodies, monoclonal F4 (anti-P-gp) and monoclonal BXP-21 (anti-BCRP), were purchased from Kamiya Biomedical (Seattle, WA). Monoclonal anti-β-actin was purchased from Sigma Chemicals (St. Louis, MO). Secondary antibody, polyclonal mouse anti-IgG with peroxidase, was purchased from BioRad ( Hercules, CA). Human P-gp membrane preparation (Human PGP Membranes) and membrane preparation lacking P-gp (Control Membrane Preparation for ABC Transporters) were purchased from Gentest (Woburn, MA). Protease inhibitor cocktail tablets were purchased from Roche Diagnostics (Mannheim, Germany); protease inhibitor cocktail for mammalian tissues was purchased from Sigma Chemicals and ABTS [2,2’-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)] was purchased from Calbiochem (La Jolla, CA). Unless specified, Western blot reagents were purchased from Amersham Biosciences (Piscataway, NJ). The Sigma alkaline phosphatase kit was purchased from Sigma Diagnostics. BCA protein assay kit was purchased from Pierce Chemicals (Rockford, IL). RNeasy Midi Kit from Qiagen (Valencia, CA) was used for RNA extraction and purification. All other reagents used in experiments were purchased from Fisher Scientific (Fairlawn, NJ).

Tissue collection and brush-border membrane preparation. Placental tissue of gestational ages 60–90 days (mean age 77 days, range 65–87 days), and 90–120 days (mean age 103 days, range 91–117 days) were collected from elective abortions (Birth Defects Laboratory, University of Washington). None of the fetuses included in the study had an abnormal karyotype. Term placenta were collected from C-sections (University of Washington Medical Center). The placenta were grouped per trimester with first trimester as 60–90 days, second trimester as 90–120 days and term. All tissue collections were approved by the Human Subjects Committee Institutional Review Board of the University of Washington. After collection, all placenta were immediately placed on ice and frozen in liquid nitrogen within an hour from collection. Syncytial microvillous plasma membrane was isolated from the human placenta by the method described by Bravo et al. (4). These membrane preparations were then subjected to Western blot analysis and ELISA.

Western blot analysis. Placental membrane preparations (20 μg of protein) were separated using 4–15% SDS acrylamide gel for P-gp and 12% SDS acrylamide gel for BCRP. Separated proteins were transferred overnight (12–14 h) at 30 V to nitrocellulose membranes. After transfer, the membranes were blocked with TBS-T (Tris-buffered saline containing 0.3% Tween 20) and 5% nonfat dry milk for 1 h at room temperature. After washing the membranes two times with TBS-T for 10 min, P-gp was detected by incubating the membranes for 1 h with a 1:1,000 dilution of the primary antibody, monoclonal F4, which reacts specifically with an epitope in the extracellular amino terminal half of the human P-gp. BCRP was detected by incubating the membranes for 1 h with a 1:200 dilution of the primary antibody, monoclonal BXP-21, which reacts specifically with an internal epitope of BCRP. The membranes were washed twice with TBS-T for 10 min and incubated for another hour with polyclonal antibody, mouse anti-IgG with peroxidase, as the secondary antibody (1:2,000 dilution). Membranes were washed again, and the signals were visualized by chemiluminescence using enhanced chemiluminescence Western blot reagents (according to manufacturer’s instructions) and imaged (Hyperfilm ECL). β-actin was used as an internal standard to confirm equal loading on the gel. β-actin was detected by incubating the membranes (1 h) with a 1:2,000 dilution of the primary antibody, monoclonal anti-β-actin, followed by a second 1-h incubation with mouse anti-IgG with peroxidase as the secondary antibody (1:5,000 dilution).

ELISA. P-gp in placental membrane preparation was quantified using the indirect ELISA. The placental membrane preparation or known standard were appropriately diluted in 50 mM carbonate buffer (pH 9.5) and coated on Immulon ELISA plate (Fisher Scientific). The plate was incubated overnight at 4°C. Then the plate was rinsed with distilled water followed by 2-h incubation with blocking buffer (containing 1% bovine serum albumin and 0.1% Tween 20 in PBS). After blocking for nonspecific binding, P-gp in the placental membrane preparations was quantified by incubating the plate for 1 h at room temperature in a 1:2,000 dilution of F4 monoclonal antibody. Horseradish peroxidase-conjugated goat anti-mouse antibody (1:5,000 dilution and incubated for 1 h) was used as the secondary binding antibody. ABTS in 1 M citric acid (pH 4.0) was used as the substrate to detect the binding antibody. A linear calibration line (0.36–5.7 μg protein) was constructed using a human P-gp membrane preparation (Human PGP Membranes) appropriately diluted in 50 mM carbonate buffer (pH 9.5). As a negative control, membrane preparation lacking P-gp (Control Membrane Preparation for ABC Transporters) was used in amounts equivalent to that used to construct the standard curve.

BCRP was quantified as described above except for the following modifications. Because no standard membrane preparation of BCRP is commercially available, a linear calibration line (0.33–5.3 μg protein) was constructed with one of the early gestational age human placental membrane preparations (gestational age: 60–90 days) as the reference, after BCRP expression in this sample was confirmed by Western blot analysis. HEK-293 cells stably transfected with empty pcDNA3 vector, which have been shown not to express BCRP (32), were used as a negative control in protein amounts equivalent to that used to construct the standard curve. The anti-BCRP BXP-21 monoclonal antibody was used at a 1:200 dilution.

The concentration of P-gp and BCRP in the membrane preparations was calculated from the appropriate calibration line. P-gp and BCRP expression in the unknown samples was normalized to the alkaline phosphatase activity of the placental membrane preparation. Alkaline phosphatase activity in all unknown samples was measured using the Sigma alkaline phosphatase kit (according to manufacturer’s instructions). Protein concentrations in all unknown and standard preparations were measured using the BCA protein assay kit.

Real-time quantitative PCR. RNA (total) was extracted from a different set of human placental tissues (60–90 days, 90–120 days, and term) using the RNeasy Midi Kit. The concentration of purified RNA was determined spectrophotometrically (SmartSpec Plus Spectrophotometer, BioRad), as was the purity, using the 260/280 absorbance ratio of 1.8 to 2.0. cDNA was prepared from 1 μg of DNase-treated total RNA using the ABI Taqman reverse transcription kit and oligo-dT primers (Applied Biosystems, Foster City, CA). Expression of P-gp, BCRP, PXR, CAR, VDR, HNF-4α, and human chorionic gonadotropin-β (hCG-β) (positive control) in the placental tissues was quantified. Quantitative real-time PCR assay of transcripts was carried out with the use of gene-specific FAM-labeled fluorescent MGB probes in an ABI 7900 Sequence Detector (Applied Biosystems). The sets of primers (5’ to 3’) and probes for P-gp, BCRP, PXR, CAR, VDR, HNF-4α and hCG-β were as follows: P-gp: probe: 6FAM-TGGCCGAATGACTGTCCCTTT-MGBNFQ; forward primer: TGGTTTGGCCTATGACGTCCTGCG; reverse primer: CCTTCTACGC- TACTGCTCCAGC; BCRP: probe: 6FAM-ATCCCAGAACGAG- CTTGG-MGBNFQ; forward primer: acttGGGCTAAAATATG- TTACTCTG; reverse primer: TTGGTCGTCAGGAGAGAGAC.
Amplification efficiency of the above primers was tested, using the validation experiment for relative quantification of gene expression (user bulletin # 2, ABI Prism Sequence Detection System, Applied Biosystems). Primers and probes were designed considering intron-exon junctions to avoid potential genomic DNA interference, as well as to exclude any nonelective amplification of similar gene products. For normalization of the mRNA data, the endogenous control β-actin was used. The primer and probe sets for human β-actin were as follows: probe: 6FAM-CGCCAGCTCACCATG-MGBNFQ; forward primer: GCACAGAACATTCCATCAT. The expression of a given target gene was determined for all placental samples in a single assay to eliminate interday variability in quantification. Therefore, if in a given assay, one of the samples did not amplify a gene (e.g., due to poor reverse transcription), that sample was discarded from the summary statistics. Each sample was assayed in triplicate. The average Ct value (cycle threshold for target or endogenous control gene amplification) was determined for all placental samples in a single assay to eliminate interday variability in quantification. Therefore, if in a given assay, one of the samples did not amplify a gene (e.g., due to poor reverse transcription), that sample was discarded from the summary statistics.

Consistent with the Western blot analysis, the results from the ELISA indicated that P-gp expression was highest in early gestational age human placenta (Fig. 2A) and placental expression of P-gp decreased dramatically with gestational age. P-gp expression, when normalized to alkaline phosphatase activity (micrograms per alkaline phosphate activity; means ± SD), in 60–90 days placenta was 44.8-fold higher (0.293 ± 0.155, P < 0.05, n = 6) than that in term placenta (0.007 ± 0.004, n = 6). Similarly, P-gp expression in 60–90 days placenta was 4.5-fold (P < 0.05) higher than that in 90–120 days placenta (0.065 ± 0.034, n = 4). However, the expression in term and 90–120 days placenta did not differ significantly (P > 0.05). P-gp expression in 60–90 days placenta, when normalized to total protein (micrograms per milligrams of protein; means ± SD), was 5.1-fold higher (75.4 ± 53.0, P < 0.05, n = 6) than that in term placenta (14.9 ± 4.7, n = 6), whereas P-gp expression in 90–120 days placenta (22.0 ± 10.0, n = 4) did not significantly differ (P > 0.05) from that in 60–90 days or term placenta. In contrast, BCRP expression in placentae was highly variable and did not change significantly with gestational age (Fig. 2B).

Subsequent to conducting the Western blots and ELISA, mRNA isolated from the preterm placentae showed some degradation of the message. Therefore, additional placentae of similar gestational ages were collected for real-time PCR quantification of P-gp and BCRP transcripts. Real-time PCR analysis of mRNA isolated from these placentae indicated expression of all the target genes (P-gp, BCRP, PXR, CAR, VDR, HNF-4α, and hCG-β) in the human placentae (Figs. 3 and 4). hCG-β transcript expression was the most abundant and decreased in the following order BCRP ≈ P-gp > PXR and CAR. Transcripts for HNF-4α and VDR were only observed in a few samples at gestational age 60–90 days and 90–120 days and not in term human placenta.

P-gp mRNA expression in early gestational age placenta, 60–90 days (n = 6) and 90–120 days (n = 6) was 6.5-fold and
5.3-fold greater \((P < 0.05)\) than that in term placentae \((n = 6)\) (Fig. 3). P-gp mRNA expression in 60–90 days placentae was not significantly different \((P > 0.05)\) from that in 90–120 days placentae. In contrast, BCRP, PXR, or CAR mRNA expression did not change with gestational age (Figs. 3 and 4). Consistent with reported data \((19, 22)\), hCG-\(\beta\) mRNA expression in 60–90 days placentae \((n = 5)\) was significantly higher \((17.2\text{-fold}, P < 0.05)\) than that in term placentae \((n = 6)\) (Fig. 4). hCG-\(\beta\) expression in 90–120 days placentae \((n = 6)\) was not significantly different \((P > 0.05)\) from that in 60–90 days or term placentae. Placental expression \((\text{mRNA})\) of hCG-\(\beta\) and P-gp were significantly correlated \((r = 0.73, P < 0.0001; \text{Spearman rank correlation coefficient})\) (Fig. 5).

**DISCUSSION**

Placental P-gp and BCRP play a significant role in preventing the entry of xenobiotics into the fetal circulation. Therefore, it is important to determine whether the expression of these proteins is dependent on gestational age. Western blot analysis of the human placental brush-border membrane showed that P-gp expression in human placentae was regulated by gestational age, but expression of BCRP was not. Because Western blots are only semiquantitative, we developed sensitive, quantitative ELISA assays to quantify P-gp and BCRP expression in human placentae of different gestational ages. Our ELISA data confirmed the Western blot data. We found
that P-gp expression was higher in early gestational age human placentae than in term placentae. Furthermore, we observed that placental expression of P-gp decreased dramatically as pregnancy proceeded and was lowest at term. This was true irrespective of whether the data were expressed with respect to alkaline phosphatase activity or total protein. The fold changes in placental P-gp expression when normalized to alkaline phosphatase activity were greater than when the data were normalized to total protein. This observation is not unexpected, given that expression of placental alkaline phosphatase (mRNA and activity) increases dramatically around the 13th wk of pregnancy and continues to increase steadily until term (21, 29). This increase likely reflects increased differentiation of cells into syncytiotrophoblasts as the placenta increases in size. Because P-gp and BCRP, like alkaline phosphatase, are expressed in the apical membrane of the syncytiotrophoblasts, expression of P-gp with respect to alkaline phosphatase activity is more appropriate.

Our data are consistent with previous findings by MacFarland et al. (by immunohistochemistry; 23) and Gil et al. (by Western blot analysis; 10). Both studies reported a gestational age-dependent decrease in expression of P-gp. Collectively, our data and the above two data sets suggest that placental P-gp expression is under developmental control. The physiological function of P-gp in the syncytiotrophoblasts remains speculative. However, with respect to xenobiotic efflux, the gestational age-dependent expression of P-gp in the syncytiotrophoblasts makes teleological sense. The fetus is at greatest danger to toxic insult from xenobiotics early in pregnancy. Therefore, it is not surprising that nature would upregulate the expression of P-gp early in pregnancy as a mechanism to protect the fetus from toxicological insult. However, the increased expression of placental P-gp early in pregnancy would imply lower fetal concentrations of drugs (P-gp substrates) that are targeted to the fetus.

Our data on real-time quantification of P-gp transcripts correspond with our ELISA P-gp protein expression data except that there is no direct correspondence between the fold changes in mRNA expression and protein expression. This lack of correspondence between protein and transcript expression is not surprising as their relative expression depends on a multitude of other factors that are independent of each other, such as the stability of the protein and transcripts. Nevertheless, the general correspondence between P-gp transcripts and protein expression suggests that the gestational age dependency of expression of placental P-gp protein is due, in part or whole, to transcriptional regulation.

The gestational age-dependent expression of P-gp, both protein and mRNA, is intriguing, and there are several possible explanations for this observation. Geick et al. (9) have reported that the PXR may be responsible for the induction of P-gp proteins in humans. PXR is a nuclear transcription factor whose ligands include steroidal hormones such as progesterone (16, 31). Ligand-bound PXR forms a heterodimer with the retinoic acid receptor and induces transcription by binding to the enhancer region of P-gp gene (3, 9). Progesterone has also been reported to increase the P-gp expression in various cell lines or animal models (2, 13, 18). Masuyama et al. reported that in pregnant mice, hepatic CYP 3A11 (mouse isof orm of CYP 3A4) message is elevated ~17–20-fold, and hepatic PXR message is elevated 20-fold, and this elevation parallels increases in the serum progesterone concentration (25). Their data suggest that elevation in serum progesterone and PXR concentrations acts in concert to upregulate expression of CYP 3A during pregnancy. In humans, it has been well established that progesterone plasma concentration increases (nearly 10-fold) during pregnancy (to ~1 μM) (36). Moreover, the placenta is the major source of pregnancy-related hormones (37). It produces large quantities of progesterone (nearly 1 mmol of progesterone per day at 40-wk gestation), and the local placental concentration of progesterone could have a major impact on regulation and expression of P-gp (5). On the basis of these reports, progesterone-mediated PXR activation may be a likely explanation for the increase in P-gp expression during pregnancy. However, Lehmann et al. (20) did not detect expression of PXR in human placenta by Northern blot analysis. Therefore, we determined whether PXR transcripts could be measured in our sample of human placentae and whether expression of these transcripts is modulated by gestational age. Simultaneously, we also determined whether placental expression of transcripts of other nuclear factors that could potentially regulate P-gp expression, such as CAR, VDR, and HNF-4α changes during pregnancy. We focused only on a select group of nuclear regulatory factors, as data in the literature indicate that cross-talk exists between these nuclear receptors that can regulate CYP 3A expression, a protein that has a regulatory machinery similar to that of P-gp (15, 24).

In contrast to the findings of Lehmann et al. (20), we observed expression of PXR transcripts in all three gestational age placentae. However, this expression was independent of gestational age, suggesting that other mechanisms exist to regulate P-gp expression in different gestational-age placentae. Similarly, expression of CAR transcripts was independent of gestational age. Interestingly, HNF-4α and VDR transcripts could be measured, albeit poorly, in only 60- to 90-day and 90- to 120-day placentae. Therefore, although we cannot rule out gestation-dependent changes in protein expression of the nuclear receptors or their translocation, our data show that en-

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**Fig. 5.** The expression of hCG-β and P-gp transcripts was highly correlated in the human placentae of varying gestational age (r = 0.73, P < 0.0001, Spearman rank correlation coefficient and r = 0.62, P < 0.0001, Pearson correlation coefficient).
hanced expression of the nuclear receptor transcripts is not responsible for the increased expression of placental P-gp observed in early gestational age placentae.

Although PXR expression did not change with gestational age, it is possible that progesterone, via PXR binding, may have produced the enhanced expression of P-gp in early gestational age placentae. However, unless progesterone acts as a suppressor of P-gp expression, such a mechanism is unlikely. This is because progesterone concentrations in maternal plasma (and presumably locally in the placentae) increase with gestational age, a pattern opposite to the observed changes in expression of P-gp.

Tachibana et al. (39) have previously suggested that a relationship may exist between the expression of the two proteins P-gp and hCG-β. Unlike progesterone and estrogen whose concentrations increase toward term, hCG is a pregnancy-specific hormone that achieves peak concentrations during the first trimester and then declines (19, 22, 36). Because this temporal pattern of expression is similar to that of our observation of placental P-gp expression, we determined whether the expression of transcripts of these two genes were correlated. We examined hCG-β because the β-subunit confers biological specificity (11, 26, 27). As expected, expression of hCG-β transcripts in the placenta decreased with gestational age. Furthermore, the expression of hCG-β and P-gp transcripts in these placental tissues was highly correlated. Although such a correlation cannot be interpreted as a cause-and-effect relationship, these data suggest that either hCG-β regulates P-gp expression in the human placenta or that a common mechanism(s) regulates the expression of both proteins.

In contrast to P-gp expression, the placental expression of BCRP, both by ELISA and Western blot analysis, was highly variable and did not demonstrate any gestational age dependency. Although this variable was lower at 90–120 days than at other gestational ages, this difference was not significant. Consistent with this observation, BCRP mRNA expression in placenta was also independent of gestational age. These data demonstrate that the observed gestational age-dependent expression of placental P-gp could not be due to a generalized decrease in expression of all proteins. Furthermore, these data suggest that regulation of placental expression of BCRP differs from that of P-gp. Not surprisingly, no correlation could be drawn between P-gp and BCRP expression in placenta of different gestational ages.

In summary, our data show that placental P-gp (protein and mRNA) expression is highest early in gestation (60–90 days) and falls dramatically at term. Thus, early in pregnancy, P-gp appears to play an important role in modulating exposure of the fetus to xenobiotics that are transported by P-gp. The mechanism(s) by which placental P-gp expression is regulated is not clear; however, an increase in transcripts of the nuclear receptor factors such as PXR, CAR, HNF-4α, and VDR is not the mechanism by which placental P-gp expression is increased in early pregnancy. Interestingly, expression of human placental hCG-β demonstrates a temporal pattern similar to that of expression of P-gp. Therefore, further studies need to be conducted to determine the role of hCG-β in the regulation of placental P-gp.

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REFERENCES


13. Kacinski BM, Yee LD, Carter D, Li D, and Kuo MT. Human placental hCG-β demonstrates a biological specificity (11, 26, 27). As expected, expression of hCG-β transcripts in the pla...


