Human choriocarcinoma cell line JEG-3 produces and secretes active retinoids from retinol

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Vitamin A (retinol) and its active derivatives (the retinoids) are essential for growth and development of the mammalian fetus. Maternally-derived retinol has to pass through the placenta to reach the developing fetus. Despite its apparent importance, little is known about placental metabolism of retinol, and particularly placental production and/or secretion of active retinoids. It has been previously considered that retinoids are recruited from the uterine environment to influence placental development and function during gestation. We have studied retinoid metabolism in the human choriocarcinoma cell line JEG-3 and demonstrate, for the first time, that active retinoids are produced endogenously by the JEG-3 cell line from retinol. These retinoids induce gene expression from a retinoic acid-responsive enhancer element reporter plasmid and modulate placental transglutaminase activity. Furthermore, retinoids are secreted from JEG-3, as shown by the activation of retinoic acid-responsive β lacZ reporter cells grown in conditioned media. These results suggest that there could be an active role for trophoblast-derived retinoids during human development.

Key words: development/ethanol/placenta/retinoic acid

Introduction

In addition to their essential roles in vision, growth and maintenance of differentiated epithelia, vitamin A (retinol) and its active derivatives, the retinoic acids (retinoids) are required for normal mammalian reproduction and fetal development (Wilson et al., 1953; Chambon, 1994). Maternal vitamin A deficiency (VAD) can result in fetal death or in a spectrum of malformations called fetal VAD syndrome (Takahashi et al., 1975; Morris-Kay and Ward, 1999). Excessive vitamin A intake can also produce a spectrum of congenital defects in a dose- and developmental stage-dependent manner (Miller et al., 1993; Morris-Kay and Ward, 1999). Since there is no de-novo fetal synthesis of retinol, the developing mammalian embryo is dependent on the maternal circulation for its vitamin A supply. The presence of measurable hepatic vitamin A stored at birth is indicative of the functionality of placental transport during gestation (Satre et al., 1992; Ross and Gardner, 1994). Despite its apparent importance, little is known about placental metabolism of retinoids. This question has been approached in animal models (sheep, mouse, monkey), but the placental metabolism of retinoids is difficult to deduce from studies in the intact animal and extrapolation of these results to humans remains uncertain (Donoghue et al., 1982; Ismadi and Olson, 1982; Vahlquist and Nilsson, 1984). Measurements of human placental, maternal and umbilical cord retinoid levels have been reported, but they shed little light on the metabolic pathway (Dimenstein et al., 1996; Sapin et al., 2000c). Using full-term human placental explants and primary cell cultures, the ability of placental tissues to produce and store retinyl esters from retinol has been demonstrated (Torma and Vahlquist, 1986; Sapin et al., 2000a).

Mammalian placenta are also known to express nuclear transcription factors [retinoic acid receptors (RARs) and retinoid X receptors (RXRs)] by which retinoid acids modulate the expression of target genes (Sapin et al., 1997; Tarrade et al., 2000). Several genes modulated by retinoids have been described in human and mouse placenta; for example, chorionic gonadotrophin hormone (CGH) (Guibourdenche et al., 1998a), placental lactogen hormone (Stephanou and Handwerger, 1995), leptin (Guibourdenche et al., 2000), receptor of epi-
dermal growth factor (Roulier et al., 1994), 17β hydroxysteroid dehydrogenase type 1 (Peltoketo et al., 1996) and STRA (stimulated by retinoic acid) genes (Sapin et al., 2000b). These data question the origin of active retinoids detected in placenta. There is no evidence for a maternal and central endocrine production during development and it is clear that plasma retinoic acid could not be the source of ligand for all placental cells (Kurlandsky et al., 1995; Bavik et al., 1997). It has recently been suggested that the uterine environment produces retinoic acid to influence early embryonic and extra-embryonic differentiation, morphogenesis and development (Zhang et al., 2000). In order to propose the placenta as an additional site of retinoid generation during development, we investigated the ability of the human choriocarcinoma cell line JEG-3 to produce and secrete active retinoids from retinol, detected by the activation of retinoic acid-responsive promoter. Furthermore, we investigated the presence of the binding protein (cellular retinol binding protein 1, CRBP1) and the two enzymes, alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH), all of which are involved in the cytosolic generation of active retinoids from retinol.

We selected the JEG-3 choriocarcinoma cell line as our trophoblastic model system. This cell line, derived from a human choriocarcinoma, appears to present many of the biological and biochemical characteristics associated with syncytiotrophoblasts, even though they are mononucleated and highly proliferative (Matsuo and Strauss, 1994). The JEG-3 cell line retains the capacity to produce progesterone, HCG (Chou, 1982), several steroids, other placental hormones and enzymes (Kato and Braunstein, 1991; Sun et al., 1998; Tremblay et al., 1999); therefore, it has been proposed as a model for placental syncytiotrophoblasts. In addition, RARα and RXRα proteins have been detected by Western blotting and immunocytochemistry as two major receptors in JEG-3 cells (Guibourdenche et al., 1998b). In this way, this cell line has been extensively used to investigate the regulation by retinoids of proliferation, differentiation and secretion of placenta cells. For all these reasons, we chose JEG-3 cells in order to answer to our question focused on the placental generation of retinoic acid from retinol. As ethanol and 4-methylpyrazole (4MP) are well known to alter the metabolism of retinoids (Xiang-Dong, 1999) and particularly the conversion of retinol into retinoic acid by acting as a competitive inhibitor for cytosolic ADH (Chen et al., 1995a,b; Duelcer, 2000), the potential effects of 4MP and ethanol on retinoid metabolism in the JEG-3 cell line were also investigated.

Materials and methods

Chemicals

All-trans retinol, all-trans retinoic acid (ATRA), trypsin, 4MP, phenylmethylsulphonyl fluoride, aprotinin, leupeptin, pepstatin, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and human purified retinol binding protein (RBP) were purchased from Sigma-Aldrich (St Quentin Fallavier, France). Horseradish peroxidase (HRP)-conjugated streptavidin, 5-(-bionanido) pentyamine and o-phenylene diamine dihydrochloride were obtained from Interchim (Montluçon, France). Culture medium [Dulbecco’s modified Eagle’s medium (DMEM), additives [glutamine, penicillin/streptomycin, dextran-coated charcoal-stripped fetal calf serum (FCS)] and lipofectamine were purchased from Life Technologie (Cergy Pontoise, France). For all experiments, ATRA and retinol were prepared as 1000× stock solution in ethanol. The maximal ethanol concentration to which the cells and tissues were exposed was <0.1%. Retinol–RBP complex was prepared and purified as previously described (Siegenthaler, 1990).

Cell culture

Human choriocarcinoma trophoblastic cells (JEG-3) and retinoids reporter cells (F9-1.8-RARβ lacZ cells) (Sonneveld et al., 1999) were maintained in DMEM supplemented with 2 mmol/l glutamine, 10 units/ml penicillin, 100 µg/ml streptomycin and 10% dextran-coated charcoal-stripped FCS (added with 0.2 mg/ml G418 for selective medium of F9-1.8-RARβ lacZ cells). No changes concerning the cellular growth and HCG production of the JEG-3 cell line were observed during 15 days treatment with dextran-coated charcoal-stripped FCS. All experiments were performed in a humidified incubator with 5% CO₂, 95% air at 37°C.

Immunocytochemistry

Antibodies against ADH and ALDH were generated using a specific antigenic amino peptide sequence conserved in all the members of each enzyme (Bavik et al., 1997). The antibody against CRBP1 was generated as previously described (Bavik et al., 1997). JEG-3 cells were grown on glass coverslips and fixed in paraformaldehyde at 4% [v/v in phosphate buffered saline (PBS)] and permeabilized using acetone. Specific cellular protein expression was checked using the primary polyclonal antibodies and a secondary antibody conjugated with HRP. HRP activity was demonstrated by using dianminobenzidine and H₂O₂ as substrate to reveal the immunoreactions.

Transfection of cultured cells

Plasmid DR5-35tk-CAT contains one copy of the retinoic acid-responsive element DR5 (direct repeat 5) ligated to an herpes simplex thymidine kinase promoter upstream of a chimeric chloramphenicol acetyl transferase (CAT) reporter gene (Mader et al., 1993). JEG-3 cells were trypsinized 16 h prior to transfection. A total of 5×10⁵ cells in 30 mm dishes were transfected using lipofectamine with 1 µg of reporter DR5-35tk-CAT plasmid and 1 µg of cytomegalovirus (CMV)–luciferase vector serving as internal control to normalize variations in transfection efficiency. The CMV-luciferase plasmid (Pharmacia, St Quentin en Yvelines, France) contains a CMV promoter and enhancer sequences that drive a luciferase (LUC) gene. Parallel transfections of the corresponding empty vectors CAT and LUC at equivalent concentrations were performed in all experiments. After an overnight incubation with DNA, cells were washed and incubated with fresh medium for an additional 12 h period. They were treated for another 24 h with retinoids in 1000× stock solutions in ethanol. In this case, the maximal ethanol concentration to which the cells were exposed was <0.1%. In other experiments, 4MP and ethanol were added at concentrations of 2 and 100 mmol/l respectively. Cell viability assays were performed for each treatment (4MP and ethanol) using MTT assays, as previously described (Godichaud et al., 2000). For this purpose, cells were seeded into 30 mm dishes at 5×10⁴ cells/well and treated for 48 h with 4MP or ethanol at concentrations ranging from 1–10 and 10–200 mmol/l respectively. During this 48 h period, a second dose of 4MP or ethanol was added after 24 h of incubation.

CAT and luciferase reporter gene assay

After incubation, cells were washed twice with PBS and treated with 700 µl of cell lysis buffer for 1 h at 4°C. The lysed cells were scraped
off and centrifuged at 950 g for 5 min at room temperature. CAT was measured by an immunoenzymatic assay (Roche Diagnostics, Meylan, France) on 100 µl of supernatant. In all studies, CAT assays were normalized to luciferase activity, determined according to the manufacturer’s instructions. The supernatants were incubated with luciferase assay reagent based on an original protocol (De Wet et al., 1987) (Promega, Madison, WI, USA). The number of relative light units was determined with a 3 s delay after a 10 s incubation.

**In-situ tissue transglutaminase (tTG) assay**

For in-situ tTG activity measurements, JEG-3 (5×10⁵ cells in 30 mm dishes) was pre-incubated with 5-(biotinamido) pentylamine and incorporation of this reagent into synthesized proteins was determined using a streptavidin–peroxidase assay following a previously described procedure (Zhang et al., 1998). The results obtained by this method are well correlated with those issued from the classical determination of tTG by using an in-vitro putrescine incorporation assay (Perry et al., 1995; Zhang et al., 1998). The activity of tTG measured in situ was calculated as a percentage of basal activity and normalized to cellular protein concentrations. Protein concentrations of the homogenates were determined using the Biuret method (Roche Diagnostics) (Camara et al., 1991).

**Retinoid activity assay in conditioned medium**

JEG-3 cells (5×10⁵ cells in 30 mm dishes) incubated overnight with retinol (with or without ethanol or 4MP) and the conditioned media were placed on the F9-1.8-RARβlacZ cells to test the ability of the media to activate the lacZ gene which is under control of a retinoic acid-responsive promoter, as previously described (Sonneveld et al., 1999). β-galactosidase was determined by using immunoenzymatic assay (Roche Diagnostics, Meylan, France). Normalization of β-galactosidase activity was performed with cellular protein concentrations, measured as described above.

**Statistical methods**

Results expressed as mean ± SD were an average value from nine values per each condition. Comparison of means was done by analysis of variance and Fisher’s t-test using the Statview II 1.03 software (Abacus Concepts Inc., Berkeley, CA, USA). For all the studies, values were considered significantly different when P < 0.05.

**Results**

In order to characterize the presence of proteins involved in the metabolic generation of retinoic acid, we first showed that JEG-3 cells presented cytoplasmic immunoreactivity for CRBP1 (Figure 1B), ADH (Figure 1C) and ALDH (Figure 1D).

The production of active retinoids by JEG-3 cells after incubation with retinol was studied by using transient transfections of a retinoid-sensitive reporter gene based on CAT expression (see Figure 2). First, we checked that the naïve JEG-3 cells (or JEG-3 cells transfected with an empty vector) did not produce CAT. CAT production by cells transfected with the DR5-tk-CAT plasmid and not treated was considered as the basal level for comparison with and detection of induction recorded following exposure to retinol.

ATRA treatment (1 µmol/l) for 24 h increased CAT response (6.5 ± 0.5-fold induction). After retinol (1 µmol/l) treatment, CAT production was significantly enhanced (3.7 ± 0.4-fold induction) but not as much as with stimulation with 1 µmol/l of retinoic acid (Figure 2). CAT production (reflecting active retinoid generation) was measured with different retinol concentrations ranging from 10⁻¹¹ to 2×10⁻⁶ mol/l. Retinol treatment increased CAT generation in a dose-dependent manner (Figure 3). The maximal CAT induction was obtained with 1 µmol/l of retinol and the half maximal induction [EC₅₀] of CAT was experimentally deduced to be 40 nmol/l of retinol (versus 5 nmol/l for retinoic acid), which is a classical value already described in enzymatic retinoic acid generation from retinol. CAT induction obtained with 1 µmol/l of retinol was similar to that which occurred with 0.01 µmol/l of retinoic acid (see Figure 2). In addition, we found that JEG-3 cells generate only the all-trans isomer of the retinoic acid from retinol (unpublished data obtained using high-performance liquid chromatography experiments).

To confirm that active retinoids were generated by enzymatic conversion from retinol, we tested potential alterations of this reaction by two well-known inhibitors of cytosolic ADH (Chen et al., 1995a,b), 4MP and ethanol, as presented in Figure 2. We first evaluated JEG-3 cells viability (using MTT assays) when they were exposed to 4MP and ethanol at usual and well described experimental concentrations. The average values of cells grown in regular medium was considered as 100% viability; no toxicity >10% was observed for 4MP concentrations between 0.5 and 10 mmol/l and for ethanol treatments between 10 and 200 mmol/l. 4MP (2 mmol/l) caused a statistically significant decrease in retinoic acid production by JEG-3 cells, causing only 54.1% of the induction observed in cells incubated with 2 µmol/l retinol alone (Figure 2). Cells treated with 100 mmol/l of ethanol also produced significantly lower amounts of retinoic acid, producing only 53.7% of retinol-treated cells. The absolute values of internal standard (not dependant upon the enzymatic conversion caused by ADH) did not change in response to 4MP and ethanol treatments (data not shown), indicating the specific effect of 4MP and ethanol on this inhibition of retinoic acid generation.

In-situ tTG is an enzymatic activity expressed by placenta and modulated by ATRA (Piccentini et al., 1992; Hager et al., 1997). In-situ tTG activity was assayed to confirm the generation of active retinoids from retinol in JEG-3 cells by using an alternative, complementary and more physiological method than the transient transfection of a reporter plasmid. As presented in Figure 4, we first demonstrated that retinoic acid repressed tTG activity in the JEG-3 cell line. Cells treated with 4MP (2 mmol/l) or ethanol (100 mmol/l) expressed the same tTG activity as the control cells, demonstrating that neither compounds modulated tTG activity in JEG-3 cells (Figure 4). Retinoic acid- (1 µmol/l) and retinol- (1 µmol/l) treated cells had statistically lower tTG activity than control cells (38 and 54% of basal activity respectively). Retinoic acid- (1 µmol/l) treated cells possessed lower tTG activity than retinol- (1 µmol/l) treated cells (Figure 4). When retinol-treated JEG-3 cells were incubated with 4MP or ethanol, tTG activity was not statistically different from that observed in control cells (Figure 4), suggesting an absence of retinoic acid generation. As with the CAT assays, the specificity of this alteration by ethanol and 4MP was assessed by the absence of alterations of absolute values on internal standards (concentrations of total cellular proteins).
Figure 1. Immunolabelling of JEG-3 cells. JEG-3 cells were seeded at 1500 cells/cm² and checked for expression of proteins involved in the retinoic acid pathway: (B) CRBP 1, (C) ADH, (D) ALDH. (A) JEG-3 cells without primary antibody (negative control). Original magnifications ×1000.

To establish the trophoblastic JEG-3 cells as a source of active retinoid secretion, we used conditioned medium assays and the F9-1.8-RARβ2 lacZ cell line as a reporter system (Sonneveld et al., 1999). Thus, retinoids present in conditioned medium will activate expression of the lacZ gene when placed on a confluent monolayer of our reporter cells. First, we demonstrated that in our short time of incubation (applied for all experiments using this cell line), retinol did not induce β-galactosidase production in F9-1.8-RARβ2 as the value was not statistically different from reporter cells incubated with ethanol of 4MP (Figure 5). Conditioned medium of JEG-3 after incubation with 1 µmol/l of retinol activated β-galactosidase generation, indicating secretion of active retinoids by JEG-3 (Figure 5). A decrease in β-galactosidase production was observed with conditioned medium from JEG-3 cells incubated with retinol and the addition of 4MP (2 µmol/l) or ethanol (100 mmol/l). β-galactosidase induction by retinoic acid was not blocked by the direct addition of 4MP (2 µmol/l) or ethanol (100 mmol/l) on F9-1.8-RARβ2 reporter cells (data not shown). These two combined results strongly suggest that the decrease in reporter cell activation by retinol-conditioned medium supplemented with 4MP and ethanol was due to a reduction of active production and/or secretion of retinoic acid by JEG-3 cells.

Discussion

Vitamin A and its active derivatives, the retinoids, are fundamental for development of the mammalian fetus (Chambon, 1994; Ross and Gardner, 1994; Morriss-Kay and Ward, 1999). The placenta plays a crucial role in the regulation of transport and metabolism of maternal nutrients transferred to the fetus. Abnormalities in these placental functions could have deleterious consequences for fetal development (Miller et al., 1993; Rossant and Cross, 2001). Little is known about human placental retinol transfer and metabolism. We previously established that human placental tissues—more precisely, the villous mesenchymal fibroblasts—are able to esterify retinol (Sapin et al., 2000a). Creech Kraft et al. have demonstrated that the early human placenta is able to metabolise 13-cis retinoic acid (Creech Kraft et al., 1989). More recently, it has been established that the all-trans retinyl acetate oxidating lipoygenase and a specific cytochrome p450 implicated in retinoid metabolism (CYP26) are present in the human placenta (Datta and Kulkarni, 1996; Trofimova-Griffin and Juchau, 1998). Together, these results indicate that the human placenta has the ability to esterify, isomerize and oxidize retinoids. Nevertheless, the generation and secretion of active retinoids from retinol by human placental cells was never investigated. To elucidate this mechanism, we chose JEG-3 choriocarcinoma...
Retinoid production and secretion by JEG-3 cells

Figure 2. Activation of a retinoic acid-responsive reporter gene by retinol in JEG-3 cells is inhibited by 4-methylpyrazole and ethanol. JEG-3 cells were transiently transfected with a retinoic acid-responsive CAT reporter gene (DR5-tk-CAT). The induction of CAT is expressed as the ratio between treated cells [retinol (Rol), retinoic acid (RA), 4-methylpyrazole (4MP) or ethanol (EtOH)] and control cells (i.e. transfected with plasmid and not treated). CAT production was determined after 24 h incubation in different conditions and normalized to luciferase activity. Each value represents the mean ± SD of the nine experiments. The different superscripts (a–f) indicate statistical differences between the different incubation conditions tested ($P < 0.05$).

Figure 3. Dose-dependent activation by all-trans retinol of a retinoic acid-responsive reporter in JEG-3 cells. JEG-3 cells were transiently transfected with a retinoic acid-responsive CAT reporter gene (DR5-tk-CAT). The induction of CAT is expressed as the ratio between (retinol) treated cells and control cells. CAT production was determined after 24 h incubation. Each value represents the mean ± SD of CAT activity normalized to luciferase activity from five experiments.

Proteins implicated in the generation of retinoic acid from retinol (such as CRBP1, ADH and ALDH) were shown to be present in JEG-3 cells. The presence of CRBP1 was strongly expected because extracts from total term human placenta are already known to express this intracellular protein which plays a pivotal role in intracellular retinol metabolism (Okuno et al., 1987; Johansson et al., 1999). For the first time, ADH and ALDH were detected in JEG-3 cells. Our results corroborate a recent study suggesting the presence of a 9-cis retinol conversion to 9-cis retinoic acid in human tissues such placenta (Paik et al., 2000). Because of the predominant presence of RXRα in mediating the biological effects of retinoids on JEG-3 cells, it has also been suggested that a possible induction of retinoic acid generation itself is a proper metabolic pathway (Guibourdenche et al., 1998b). Our present work shows, for the first time, that derived trophoblastic cells are able to produce and secrete retinoids at functional levels from retinol. Two mammalian placentas have also been shown to produce retinoic acid from retinol: the porcine (Parrow et al., 1998) and the mouse (yolk sac) placenta (Bavik et al., 1997).

Due to the metabolic property demonstrated in this study, the JEG-3 cell line belongs to a little group of transformed and established cell lines experimentally shown to be able to generate active retinoids from retinol; this group includes the pig kidney cell line LLC-PK1 (Napoli, 1986), the rat hepatic stellate cell line HSCT6 (Vogel et al., 2001) and the human intestinal cell line Caco-2 (Lampen et al., 2000). For each determination, induction obtained with retinol was always less than with all-trans retinoic acid exposure. The enzymes involved in retinol metabolism, such as ADH and ALDH, are saturable (Leo et al., 1987; Lindahl, 1992; Duester, 2000). Moreover, intracellular retinol can be metabolized following different pathways (oxidation but also decarboxylation,
Figure 4. Decrease of tissue trans-glutaminase (tTG) activity by retinol in JEG-3 cells is inhibited by 4-methylpyrazole and ethanol. In-situ tTG activity was assayed after 24 h incubation in the described conditions and was normalized by intracellular protein concentrations. For each parameter, the value represents the mean ± SD from nine experiments. Different superscripts (a–d) indicate statistical differences between tissue transglutaminase activity values obtained from control cells and from treated cells (P < 0.05).

Figure 5. JEG-3 cells are able to secrete functional retinoids from retinol. JEG-3 cells were incubated overnight with retinol (added or not with ethanol or 4-methylpyrazole) and the conditioned media (JEG-3CM) were placed on the F9-1.8-RARβ-lacZ cells to test its ability to activate the lacZ gene. β-galactosidase was determined by using immunoenzymatic assay and normalized with cellular protein concentrations. For each parameter, the mean ± SD from nine experiments are presented. Different superscripts (a–d) indicate statistical differences between β-galactosidase activity values obtained from control and treated cells (P < 0.05).

conjugation or epoxydation). Together, these two reasons could explain why retinol is only partially used to form retinoic acid.

What could be the biological function of active retinoids formed and secreted in this derived trophoblastic cell line, JEG-3? In-situ retinoid production is a single and efficient way to regulate cellular proliferation, differentiation and adhesion. It has been well described that retinoids stimulate differentiation of JEG-3 cells (indicated by the rate of HCG secretion) but consistently reduce, in a dose- and time-dependent manner, the proliferation and the adhesion of these cells to the endometrial
Retinoid production and secretion by JEG-3

In addition, regulation of TGF by retinoic acid in JEG-3 cells could also participate in the regulation of apoptosis, a cellular event indispensable for the harmonious development of this cell line (Hager et al., 1997; Autuori et al., 1998). In-situ generated retinoids could also be implicated in the implantation process. A previous study has demonstrated that RARα/RXRα (a functional heterodimer of nuclear receptors) is present in the JEG-3 cell line, in the proliferative intermediate trophoblasts and in the invasive extravillous trophoblasts during invasion process (Tarrade et al., 2000). Upon retinoic acid treatment, a rat choriocarcinoma cell line was shown to irreversibly lose connexin31 gene expression, a molecule strongly implicated in the implantation process (Grummer et al., 1996). Other placental events could also be regulated by retinoids in the JEG-3 cell line via the modulation of expression of retinoic acid-sensitive genes (in terms of transcripts and proteins). These could include the following (i) hormone production: total HCG and its subunit α and β (Kato and Braunstein, 1991; Matsuo and Strauss, 1994; Yamada et al., 1997); (ii) metabolism and transfer: an increase in gp330/megalin (a membrane-bound 550 kD calcium binding glycoprotein belonging to the low density lipoprotein receptor family) mRNA expression has been seen in JEG-3 cells cultured with retinoids (Liu et al., 1998); and (iii) steroidogenesis: progesterone production by the JEG-3 cell line is stimulated by retinoic acid, without changes of intracellular levels of cAMP (Kato and Braunstein, 1991). In addition, key enzymes of steroidogenesis are regulated by retinoic acid in the JEG-3 cell line. These include 11β-hydroxysteroid dehydrogenase type 2 mRNA expression and activity (Tremblay et al., 1999), 17β-hydroxysteroid dehydrogenase type 1 mRNA expression and activity (Piao et al., 1997) and CYP19 (p450 aromatase) regulated by a region upstream of exon 1 (Sun et al., 1998).

Extrapolating our findings on choriocarcinoma cells to normal trophoblast function, our data point to a possible linkage between the nutrient supply of retinol to the placenta, and the generation of strong developmental morphogene and gene regulation and placental gene regulation and physiology. During pregnancy, placental cells may be exposed to deleterious maternal conditions including alcohol abuse. Links have been established between alcohol abuse, fetal malformations [the fetal alcoholic syndrome (FAS)] and alterations of retinoid metabolism (Zachman and Grummer, 1998; Leo and Lieber, 1999). Previous studies and our present work have demonstrated the interference of alcohol on synthesis of functional retinoids from retinol (Xiang-Dong, 1999). We suggest that alterations of placent retinoid metabolism by maternal ethanol ingestion could provide a novel and additional explanation for the genesis of FAS pathology, by alteration of placental physiology. A molecular explanation due to the heterodimerization of RXR with peroxisome proliferator activated receptor (PPARγ) could be also proposed in the same way. In fact, a recent study demonstrated that PPARγ expression and activity is indispensable for harmonious placentation (Barak et al., 1999). Alteration of retinoic acid generation from retinol in placental cells could consequently alter the functionality of the PPARγ/RXR heterodimer, indispensable for trophoblastic invasion (Tarrade et al., 2001) and JEG-3 physiology (Matsuo and Strauss, 1994). It is well established that peroxisome proliferators and retinoids differentially regulate JEG-3 cell endocrine activities, suggesting that JEG-3 cells possess mechanisms to respond to nutrient cues (active retinoids and/or peroxisome proliferators) using the activation of the PPARγ/RXR heterodimer, and this could be altered by toxins such as ethanol.

In conclusion, our study establishes that a human-derived trophoblastic cell line produces and secretes active retinoids from retinol. It is well established that retinoids regulate proliferation, differentiation and target gene expression in trophoblastic and uterine cells. These results suggest that endogenous and trophoblast-derived retinoids could have active roles in human early development. By blocking this metabolic pathway, maternal ethanol consumption may have deleterious effects during placental implantation and development.

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Retinoid production and secretion by JEG-3


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