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Sterylsulfatase Expression in Normal and Transformed Human Placental Cells

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Isolated cytotrophoblast cells and choriocarcinoma cell lines are commonly applied *in-vitro* systems for the study of human placental endocrine function. We tested these normal and transformed placental cells for expression of the enzyme sterylsulfatase which is necessary for the production of free steroids from sulfoconjugated precursors in the placenta as well as in other human tissues, and compared the results with respective data obtained from term placental tissue. Specific sterylsulfatase activity was highest in placental homogenates but was lower by about a factor of 5 to 10 in homogenates of freshly isolated cytotrophoblast or JEG-3 cells and by about a factor of 100 in BeWo cell homogenates; the enzyme activity could not be detected in Jar cells. Sterylsulfatase mRNA levels as analyzed by Northern blotting roughly paralleled the levels of enzyme activity measured in cytotrophoblast, JEG-3, and BeWo cells; in Jar cells, RNA species complementary to the specific probe were clearly detectable but differed by size from the mRNA species found in the other cells. Our results indicate that sterylsulfatase activity is differently expressed in normal and transformed placental cells due to different rates or products of gene transcription in these cells.

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INTRODUCTION

Steroid sulfates, the major metabolites of steroids in human blood and certain tissues, fulfil contrasting biological functions by acting both as metabolic end products destined for excretion and as intermediates which are further metabolized to distinct sulfoconjugated as well as to free steroids [1–3]. To enter the latter pathway, steroid sulfates must gain access to and be hydrolyzed by the membrane-bound enzyme sterylsulfatase (STS; EC 3.1.6.2) [4, 5]. In man, sterylsulfatase activity is highest in the placenta where it is essential for the production of huge amounts of estrogens from sulfoconjugated precursors of fetal and maternal origin [3, 6]. Low but significant STS activities, however, have been found in numerous other tissues where they likewise may be involved in the local production of free and possibly active steroids [7, 8].

The sterylsulfatase is coded by a single gene that has been mapped to the distal short arm of the human X chromosome (Xp22.32) near the pseudoautosomal region [9, 10]. Like a few other genes, the STS locus partially escapes from X-inactivation, resulting in a dosage inequity between XX and XY cells [9]. Other features of the regulation of STS expression in trophoblastic as well as in non-trophoblastic human tissues, however, are largely unknown. In the present study, we analyzed the sterylsulfatase activity and the level of specific mRNA in normal (isolated cytotrophoblast [11]) and in transformed placental cells (BeWo [12], Jar [13] and JEG-3 [14] choriocarcinoma cell lines) in order to select appropriate *in-vitro* systems for future studies of human sterylsulfatase expression [15].

EXPERIMENTAL

Cell culture

Cytotrophoblast cells were isolated from term placentas and were cultured for up to 24 h according to Kliman *et al.* [11] with the modifications described in Ugele *et al.* [16]. Choriocarcinoma cell lines BeWo, Jar and JEG-3 obtained from the American Type Culture Collection (Rockville, MD, U.S.A.) were grown in plastic culture flasks at 37°C in 95% air, 5% CO_2 humidified atmosphere using Ham's F-12 medium supplemented with 15% fetal calf serum, 2 mmol/l

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glutamine, 100 IU/ml penicillin, 100 μg/ml streptomycin sulfate. Prior to the experiments, the choriocarcinoma cells were plated on 90 mm (2–4 × 10^6 cells) or 60 mm culture dishes (0.8–1.3 × 10^6 cells), for RNA analysis or determination of STS activity and hCG production, respectively; medium was changed once after 48 h. Confluent cells were harvested after 3 days and were either processed for RNA extraction or homogenized. To measure the production of hCG, aliquots of the cells were further incubated for 24 h with fresh medium.

Determination of specific STS activity

Sterylsulfatase activity was measured in homogenates of term placental tissue [17] and of placental cells [16] using 10 μmol/l ^{35}S-labeled dehydroepiandrosterone sulfate (DHEA-S) as the substrate [17]. Protein concentration of the various homogenates were measured according to [18] and were adjusted to 1 mg per ml prior to assaying STS activity.

hCG assay

After the additional 24 h incubation period, medium (6 ml) was aspirated and the cells were washed once with 2 ml phosphate buffered saline (PBS), scraped off and processed for the determination of cellular protein. hCG was quantitated in the medium and in the wash solution by an automated enzyme immunoassay calibrated against the 1st IRP 75/537 (IMx analyzer, Abbott Diagnostika, Wiesbaden, Germany).

RNA analysis by Northern blotting

STS cDNA was obtained from American Type Culture Collection (No. 59322, [19]). A 338 bp fragment (corresponding to nucleotide sequence 779–1117 of the STS cDNA [20]) was cloned in pBluescript and was transcribed *in vitro* into labeled RNA using digoxigenin–UTP and T7 or T3 RNA polymerase, respectively. Similarly, a digoxigenin-labeled RNA probe was prepared from a 357 bp fragment (nucleotide sequence 324–681) of γ-actin cDNA [21]. Total RNA was extracted from cells [22] or placental tissue [23] and was quantified photometrically at 260 nm. About 10 μg RNA dissolved in 10 mmol/l 3(*N*-morpholino)propanesulfonic acid, 4 mmol/l sodium acetate, 0.5 mmol/l EDTA, pH 7.0, 50% (v/v) formamide, 6.5% (v/v) formaldehyde were heated for 15 min at 65°C, cooled on ice, separated by electrophoresis in 0.9% agarose containing 0.25 mol/l formaldehyde and transferred to nylon membrane by electroblotting. The RNA was fixed to the membrane by UV crosslinking (20 s) followed by baking at 80°C (2 h). Hybridization with STS and γ-actin specific RNA probes at concentrations of 50 and 10 ng/ml, respectively, as well as washing of the membrane were carried out at 68°C using stringent salt concentration [24]. Labeled RNA was detected by a chemiluminescence technique applying anti-digoxigenin antibody conjugated with alkaline phosphatase and AMPPD [3-(2′-spiroadamantane)-4-methoxy-4-(3″-phosphoryloxy)-phenyl-1,2-dioxetane] as substrate [24]. All solutions used for RNA analysis were treated with diethylpyrocarbonate.

RESULTS

The transformed cell lines studied proved to be of placental origin by their ability to secrete hCG during culture [JEG-3: 4.6 ± 1.2, BeWo: 1.8 ± 1.5, Jar: 6.2 ± 0.7 IU × (mg cell protein)$^{-1}$ × (24 h)$^{-1}$ (MW ± SD, $n = 2$)]. Cytogenetic analysis revealed the known karyotypic features of these cell lines: chromosomal polymorphism, chromosome numbers in the triploid to hypotetraploid range and the presence of both an X and a Y chromosome [25]. Homogenates prepared from the transformed cells, from freshly isolated normal cytotrophoblast cells and from term placental tissue revealed strikingly different sterylsulfatase activities (Table 1). Specific STS activity was highest in placental homogenates but was 5- to 10-fold lower in homogenates of cytotrophoblast cells [difference statistically significant, $P < 0.001$ for both male and female samples (unpaired t-test)]. STS activity values found in JEG-3 cells resembled respective values measured in male cytotrophoblast cells ($P > 0.1$); as compared to JEG-3 cells, STS activity was about fifteen times lower in BeWo cells ($P < 0.001$) and was below the limit of detection in Jar cells ($P < 0.001$).

Using a digoxigenin-labeled STS-specific antisense RNA probe, two mRNA bands of about 2.7 and 5.2 kb size were detected by Northern analysis in total RNA samples prepared from term placental tissue and from cytotrophoblast cells (Fig. 1); with a few RNA preparations, a faint band of about 7 kb size was seen additionally. The specificity of RNA–RNA hybridization was demonstrated by the absence of any labeled band on the blot when total RNA from a STS-deficient placenta [sp. act. ≤ 1 pmol min^{-1} (mg protein)$^{-1}$] was analyzed using the STS-specific RNA probe (Fig. 1). The same pattern of mRNA bands as found with RNA from cytotrophoblast cells was also seen when JEG-3 or BeWo cell RNA was tested; however, the relative amount of specific STS gene transcripts was rather low

Table 1. Specific sterylsulfatase activities measured radiometrically in homogenates of placental tissue and trophoblast-derived cells using ^{35}S-labeled DHEA-S as the substrate (mean ± standard deviation of n independent preparations)

Homogenate of	Sterylsulfatase activity [pmol × min^{-1} × (mg protein)$^{-1}$]
Term placenta (female fetus)	1480 ± 641 ($n = 5$)
Term placenta (male fetus)	849 ± 225 ($n = 7$)
Female cytotrophoblast cells	261 ± 67 ($n = 8$)
Male cytotrophoblast cells	144 ± 42 ($n = 7$)
JEG-3 choriocarcinoma cells	110 ± 25 ($n = 5$)
BeWo choriocarcinoma cells	7.9 ± 3.8 ($n = 4$)
Jar choriocarcinoma cells	≤1 ($n = 4$)

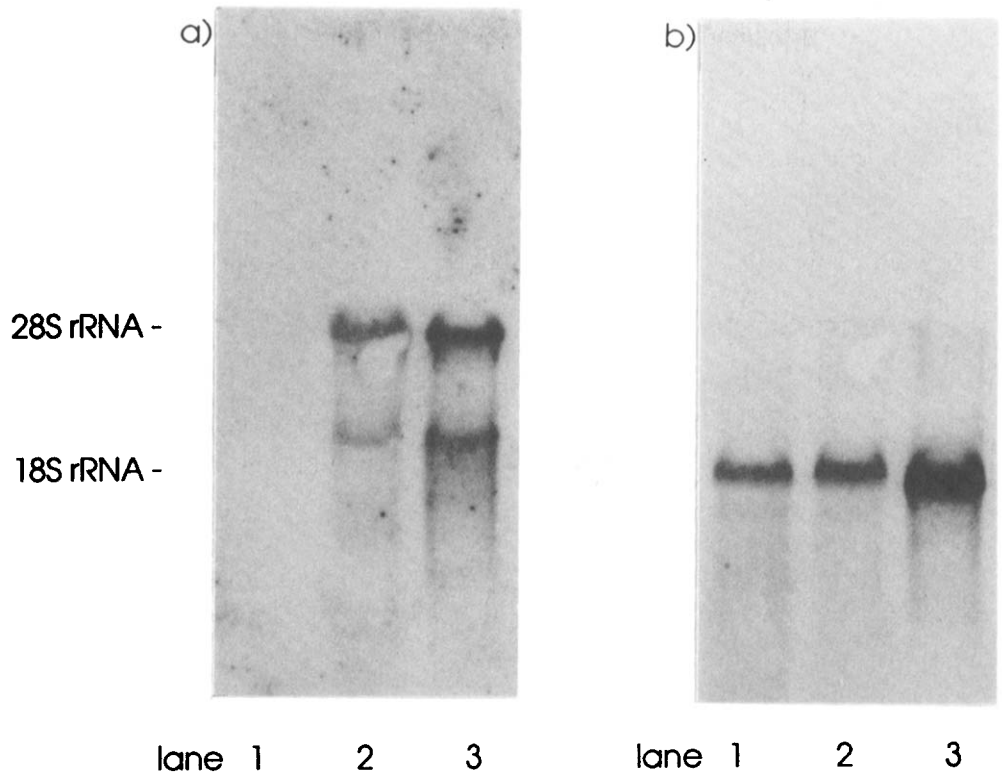

Fig. 1. Specificity of STS mRNA probe hybridization. Total RNA prepared from a STS-deficient male placenta stored 6 weeks at −70°C (10 µg, lane 1), from a normal male placenta stored for 5 months at −70°C (9 µg, lane 2) and from cytotrophoblast cells isolated from a female placenta and kept in culture for 17 h (11 µg, lane 3) were electrophoresed on 0.9% agarose gel, blotted on nylon membrane, hybridized with digoxigenin-labeled antisense RNA specific for STS (a), washed and re-hybridized with digoxigenin-labeled antisense RNA specific for γ-actin (b). For comparison, the migration of 28S (~5.1 kb) and 18S (~1.9 kb) ribosomal RNA during electrophoresis is indicated.

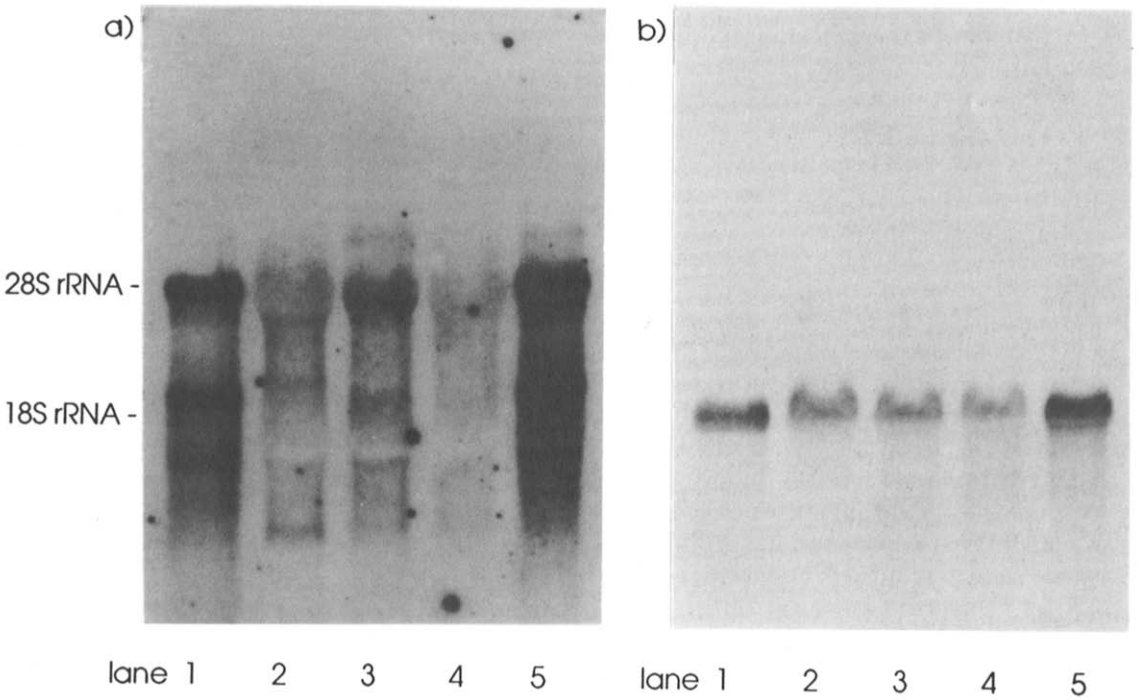

Fig. 2. Northern analysis of RNA from normal and transformed placental cells. Total RNA from male cytotrophoblast cells (10 µg, lane 1), from choriocarcinoma cell lines Jar (12 µg, lane 2), JEG-3 (11 µg, lane 3), and BeWo (10 µg, lane 4) as well as from male term placenta (12 µg, lane 5) were analyzed using the STS (a) or γ-actin probe (b). For experimental details see Fig. 1.

especially in RNA samples from the latter cell line. In contrast to these results, Northern analysis of RNA from Jar cells revealed two labeled RNA bands (about 3.0 and 5.0 kb) which differed by size from the STS gene transcripts found in the other normal or transformed placental cells (Fig. 2). All cells and tissues studied expressed a single γ-actin mRNA species of about 2.1 kb length (Figs 1 and 2).

DISCUSSION

A recent report on sterylsulfatase immunocytochemistry and *in situ* hybridization localized the enzyme as well as its mRNA exclusively to the syncytiotrophoblast of human placenta during different periods of pregnancy [26]. In an earlier immunohistochemical study, we likewise observed STS immunoactivity primarily in the syncytiotrophoblast of human term and preterm placenta but found low amounts of the enzyme also in cytotrophoblast cells underneath the syncytium [27]. The present results corroborate our earlier findings in as far as they clearly indicate that all or at least a significant fraction of the cytotrophoblast cells isolated from term placenta and cultured <24 h (at what time they still appeared as mononucleated nonsyncytial cells [16]) express both STS activity and mRNA. However, taking into account that specific STS activities of homogenates from cytotrophoblasts and from placental tissue differ by about a factor of 5 to 10 (Table 1), that our cytotrophoblast preparation is roughly 90% pure, and that the syncytiotrophoblast represents only about 15% of the mass of term placental tissue [28], the specific enzyme activity apparently is 30- to 40-fold higher in the syncytiotrophoblast as compared to cytotrophoblast cells.

The choriocarcinoma cell lines BeWo, Jar, and JEG-3 are known to share several basic features of placental steroid metabolism with the normal trophoblast: they all produce progesterone at a similar rate, lack 17α-hydroxylase activity, and are able to aromatize exogenous C19-steroids [15]. In addition, JEG-3 cells have already been shown to be able to produce estrogens from sulfoconjugated C19-steroids, although at a lower rate as compared to unconjugated substrates [15, 29]. Our results now indicate that JEG-3 cells resemble cytotrophoblast cells with respect to their specific STS activity. Moreover, in both JEG-3 and the nontransformed placental cells STS mRNA molecules are detectable which cannot be distinguished electrophoretically from each other or from specific mRNA isolated from whole placenta. In BeWo and in Jar cells, however, STS activity turned out to be rather low or even lacking, respectively; in accordance with these findings, the latter transformed cells contained either very low relative amounts of STS mRNAs (BeWo) or gene transcripts which differ from the normal STS mRNAs by size and therefore possibly may not be translated into protein or may code for an inactive enzyme (Jar). It thus is concluded that none of the choriocarcinoma cell lines tested exhibit STS activity at a level as high as the one present in the syncytiotrophoblast of human term placenta; while, however, the level of STS gene expression by JEG-3 cells at least approximates the one of term placental cytotrophoblast cells, BeWo and Jar cells have lost the placenta-specific ability to strongly express the sterylsulfatase activity.

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