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Specific detection of type II human chorionic gonadotropin beta subunit produced by trophoblastic and neoplastic cells

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ABSTRACT

Background: The sequence of the beta-subunit of human chorionic gonadotropin (hCGβ) varies depending on whether hCGβ is encoded by type I or type II genes. Type II genes are upregulated in trophoblast and cancer but hCGβ can be detected in the serum of nonpregnant women and healthy individuals. We aimed to determine whether monoclonal antibody (mAb) FBT11-II specifically detects hCGβ encoded by type II genes (type II hCGβ).
Methods: Competitive inhibition assays with synthetic peptides, immunocytochemical and immunohistochemical studies, type II hCGβ dosing immunoassays and sequencing of *CGB* genes were performed.
Results: Competitive inhibition assays determined that mAb FBT11-II recognizes the type II hCGβ derived peptide. CGB mRNA sequencing of JEG-3 (trophoblastic) and T24 (bladder) cell lines confirmed that JEG-3 expresses type II genes while T24 expresses exclusively type I. FBT11-II only recognizes JEG-expressed hCGβ. Placenta immunohistochemical studies confirmed that type II hCGβ expression is restricted to the syncytiotrophoblast. Immunoassays detected type II hCGβ in serum of patients with either nontrophoblastic cancers or fetal Down syndrome.
Conclusion: Type II gene expression can be detected using FBT11-II. This specific recognition could improve the clinical usefulness of assays aimed at either managing aggressive tumors or screening for Down syndrome.
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1. Introduction

Human chorionic gonadotropin (hCG) is a member of the glycoprotein hormone family, which also comprises LH, FSH, and TSH [1]. These hormones share a common α-subunit of 92 amino acids that is non-covalently associated with a hormone β-subunit. The β-subunit of hCG (hCGβ) contains 145 amino acids. In addition to its expression by trophoblastic cells during pregnancy, hCGβ is produced by normal tissues of different histological origins and is expressed by gonadal and nongonadal neoplasms [2,3]. While the hCG α-subunit (hCGα) is encoded by one gene on chromosome 12q21.1-23 [4], hCGβ is encoded by six non-allelic genes (*CGB* genes) clustered on chromosome 19q13.3 and named *CGB1* or β1, *CGB2* or β2, *CGB3* or β3, *CGB5* or β5, *CGB7* or β7 and *CGB8* or β8 [5–7]. Recent data show that the diversity of these genes is one of the highest reported for human genes and that high interindividual and intergenic differences in expression exist [7,8]. Genes β1 and β2 might encode a protein unrelated to hCG [9] while the remaining four genes encode the same protein, with the exception of the β7

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gene which encodes for a protein with an alanine at position 117 as opposed to an aspartic acid in the other three genes [6,10,11]. On the basis of the amino acid residues in position 117, genes encoding the hCGβ subunit were classified as type I genes if they encoded an alanine (of which only one exists: gene β7) or as type II genes if they encoded an aspartic acid (of which three exist: β3, β5, β8) [12]. Numerous normal nontrophoblastic tissues express preferentially type I gene, whereas, in addition to type I gene, normal pituitary, testis and trophoblast as well as malignant trophoblastic and malignant nontrophoblastic tissues of different histological types express type II genes [2]. These differences in expression offer the possibility of distinguishing between hCGβ expressed by most normal nontrophoblastic tissues (i.e. type I) from hCGβ produced only by normal trophoblast and by malignant cells (i.e. type II).

Studies to differentiate between type I and type II genes have concentrated on elegant techniques using molecular beacons or nested PCR and are able to detect a single nucleotide difference at position 117, i.e. GCC as opposed to GAC coding respectively for alanine or aspartic acid [2,13]. However, depending upon the techniques employed, different results were observed in tissues and in cell lines such as the T24 cell line [2,13]. On the other hand, a specific antibody able to distinguish between the free hCGβ subunits transcribed and translated from either type I or type II genes has never been described: fusion experiments using synthetic peptides analogous to the 114–122 region of hCGβ as immunogens and aimed at generating monoclonal antibodies capable of distinguishing an aspartic acid from an alanine at position 117 have been unsuccessful, at least in our laboratory.

In the present study, we show that mAb FBT11-II, which is specific to free hCGβ and recognizes the nicked form of this subunit [14,15] is capable of discriminating between hCGβ subunits encoded by type I and type II genes. Interestingly, the ELSA-FbHCG immunoradiometric assay is based on FBT11, the parental clone of FBT11-II, which suggests that this assay only measures type II hCGβ expression. This result has an impact on studies that use this assay since only hCG produced during either pregnancy and malignant processes will be preferentially measured. hCG produced by normal non-trophoblastic cells will be undetected.

2. Materials and methods

2.1. Subjects

Sera from pregnant women with fetal Down syndrome (n = 9) were obtained from the serum library of Hopital Cochin, Paris. These blood samples had been collected during the first trimester of pregnancy. Sera from patients with lung cancer (n = 30) or bladder cancer (n = 3) were obtained from the serum library of Institut Curie, Saint-Cloud. These blood samples had been collected according to protocols previously approved by the human studies committee of each institution. An informed consent had been obtained from each participant.

2.2. Cell lines

Human choriocarcinoma cell line JEG-3 was cultured in Eagle's Minimum Essential Medium and human bladder carcinoma cell line T24 was cultured in Dulbecco's MEM (4.5 g/l glucose). All media were supplemented with 10% fetal calf serum and 1× penicillin–streptomycin (Invitrogen, Cergy Pontoise, France). Cell culture supernatants from confluent JEG-3 or T24 cell lines were concentrated 10× using amicon ultra-15 centrifugal filter units (nunc, Thermo Fisher Scientific, Brebières, France).

2.3. Solid-phase peptide synthesis

Synthetic 7-mer peptides corresponding to residues 1 through 7 of the hCGβ subunit were synthesized as previously described [16] by the solid-phase method [17] in an Applied Biosystems Model 430 A peptide synthesizer. The sequences of the peptides were as follows: SKEPLRP (corresponding to residues 1 through 7 of hCGβ encoded by type II genes β3, β5 and β8); SREMLRP (corresponding to residues 1 through 7 of hCGβ encoded by type I gene β7); and SREPLRP (corresponding to residues 1 through 7 of the LHβ gene).

2.4. Antibodies

Monoclonal antibodies (mAbs) FB09, FB12 and FBT11-II were obtained as previously described [16–18]. MAbs FB09 and FB12, elicited against a synthetic peptide analogous to the COOH 109-145 terminal portion (CTP) of hCGβ, are directed against the 134–140 and 110–116 regions, respectively [18]. These mAbs are specific for either hCG or its hCGβ subunit and do not bind to LH or its LHβ subunit. MAb FBT11-II, elicited against purified hCGβ subunit (CR 129), is directed to a discontinuous epitope encompassing residues 1 through 7 and 82 through 92 of hCGβ [16]. FBT11-II is an IgG1 specific for the hCGβ subunit and does not bind to hCG nor to LH while its cross reactivity with the LHβ subunit is of 0.6% [14,16]. Mouse IgG1 isotype control MG100 (Invitrogen, Cergy Pontoise, France) was used as a control antibody. Polyclonal antibody A0231 from Dako (Trappes, France) was raised against the isolated beta-chain of hCG and reacts with free hCGβ and dimeric hCG.

2.5. Competitive inhibition assays with peptides

Competitive inhibition assays were performed as previously described [16]. Briefly, ^{125}I labeled hCGβ, containing both type I and type II hCGβ, (NIH-hCGβ (CR-125) labeled by the IODO-GEN method [19]) was employed as the tracer. All experiments were performed in 50 mM phosphate buffer, pH 7.5, containing 154 mM NaCl, 0.02% sodium azide, and 1% bovine serum albumin. First, we determined the dilution of FBT11-II which produced a 50% binding to ^{125}I-hCGβ (30,000 cpm) in the absence of peptide. Then, competitive inhibition assays were performed with the defined dilution of antibody. Briefly, displacement curves were generated in the presence of increasing concentrations of unlabeled peptides as follows: 100 μl of ^{125}I-hCGβ, 100 μl of monoclonal antibody, and 50 μl of the competitive inhibitor (at increasing concentrations) were incubated simultaneously at 4 °C for 18 h. The antigen–antibody complex was then precipitated by adding normal human serum diluted (1:3) in phosphate buffer (100 μl) and 1 ml of 20% polyethylene glycol. After centrifugation, radioactivity of the pellet was measured. Dose–response curves showed a half-maximal inhibitory dose for each molecule tested (ID_{50}).

2.6. Sequencing

RNA obtained from human bladder carcinoma cell line T24 was reverse-transcribed into cDNA with 400 units of SuperScript II RNase H-reverse transcriptase (Life Technologies, California, USA). Two microliters of this cDNA was used for 35 cycles of polymerase chain reaction (PCR) with 1.25 units of AmpliTaq Gold from Applied Biosystems (Courtaboeuf, France) with the CG Forward and CG Reverse primers [20] to obtain the CGB insert. The PCR products were purified by electrophoresis on 1% agarose gel using the S.N.A.P. gel purification kit from Invitrogen (Cergy Pontoise, France). Next, the inserts and pcDNA3 plasmid (Invitrogen) were digested with XbaI from New England Biolabs (Frankfurt, Germany) overnight at 37 °C and 19 pmol of plasmid was dephosphorylated using 0.4 units of calf intestinal alkaline phosphatase from Promega (Charbonnières-les-bains, France) following the manufacturer's instructions. After precipitation and ligation of the digested products, sequences were cloned using the TOP10F' chemically competent *E. coli* from Invitrogen following the manufacturer's instructions. Fragments were directly sequenced with sequencing primers T7 and Sp6 together with the hCG Forward and hCG Reverse primers using the ABI PRISM Dye Terminator Cycle Sequencing Reaction Kit (PE Biosystems, Courtaboeuf, France) on an

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