Expression of beta-human chorionic gonadotropin in ovarian cancer tissue

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Summary

The purpose of the present investigation was to determinate expression of human chorionic gonadotropin gene in ovarian cancer tissue. The study included 15 patients with epithelial ovarian carcinoma. The expression of mRNA *hCGβ* was determinated by the RT PCR method and the distribution of the hormone in study tissue was analyzed immunohistochemicaly. In all 15 study specimens of the ovarian carcinoma tissue the active *hCGβ* gene was found, whereas noncancerous tissue demonstrated lack of the hormone expression. Thus, the study clearly shows that the expression of *hCGβ* is the feature of ovarian cancer tissue.

Key words: hCGβ; mRNA of hCGβ; Ovarian cancer.

Introduction

Human chorionic gonadotropin (hCG) is a sialoglycoprotein hormone composed of two noncovalently linked subunits - α (hCGα) and β (hCGβ) [1]. Physiologically, hCG is produced by syncytiotrophoblastic cells of the placenta, however precise biological significance of hCG in fetus development is still unknown [2]. Human chorionic gonadotropin is also secreted by malignant trophoblastic tumors. In gestational trophoblastic disease, hCG is approved as an established tumor marker [3]. Recent studies have demonstrated that besides the placenta and malignant trophoblastic disease, varieties of tumors of different origin secret hCG and especially its β-subunit [3-5]. The serum from patients with non-trophoblastic cancers, such as cancers of the ovary, cervix, gastrointestinal tract, bladder, lung, and breast revealed hCG and hCGβ immunoreactivity [6-11]. Acevedo and co-workers have shown that the presence of membrane-associated hCG is a common phenotypic characteristic of cancer [12, 13]. The role of hCG in tumorigenesis is unknown but recent reports suggest that hCGβ can stimulate growth of cancer cells or inhibit the apoptosis and that elevated serum level of hCGβ correlates with more aggressive cancer and is resistant to the therapy [14, 15]. Until now there is no evidence of human chorionic gonadotropin in gynecological cancer tissue, however the serum immunoreactivity of hCG and hCGβ has been previously observed in patients with gynecological malignancies [16]. Also the urinary hCGβ core-fragment is present in patients with cancer of the ovary, cervix and endometrium [17-19].

The aim of the present study was to determinate expression of the human chorionic gonadotropin gene in ovarian cancer tissue.

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Material and Methods

Tissue samples

Ovarian cancer samples. Surgical specimens of tissue were obtained from 15 patients with epithelial ovarian carcinoma (median age 55, range 40-69) treated by surgery at the Department of Gynecologic Oncology, Poznań University of Medical Sciences in 2002. In all patients, histological proof, including tumor grading, was obtained and the staging was performed according to the International Federation of Gynecology and Obstetric (FIGO). Histology groups presented as follows: ovarian cancer of serous type (10 cases, tumor grading G1 – 3, G2 – 4, G3 – 3; FIGO IA – 1, IC – 2, IIC -1, III – 5, IV - 1), ovarian cancer of mucinous type (1 case, tumor grading not determined, FIGO - III), clear cell type (2 cases, tumor grading G1 and G2, FIGO IC and III), and solid type (2 cases, tumor grading G3; FIGO III).

Control samples. The control samples used to evaluate hCGβ in carcinoma tissue represented material derived from the same patients, originating from operation materials which did not possess any pathological changes in macro- and histopathological investigations. The control material of the endometrium (5 samples) and the uterine cervix (5 samples) were collected. Four samples of placentas from term delivery served as a positive control.

The samples taken at the operation were frozen in liquid nitrogen. No patients received chemotherapy or radiotherapy prior to surgery.

RNA extraction and cDNA synthesis

Total RNA was isolated from the tissue of gynecological carcinomas and control samples – placenta, myometrium, endometrium and uterine cervices with TRIZOL reagent (GIBCO BRL, Grand Island, New York, USA), according to the manufacturer's protocol. About 10 μg of RNA (DNase treated) was employed individually for one reverse transcription reaction in a mixture containing: 50 pmols of hCGβ sequence specific primer (5'-GAGAAGCCTTTATTGTG-3', nucleotides: 506-522, PubMed – AC J00117) or 100 pmols Oligo $(dT)_{10}$

primer, 5 U/µl Expand reverse transcriptase, 1x Expand reverse transcriptase buffer, 1 mM of dNTPs, 20U RNase Inhibitor, 10 mM DDT. The reaction mixture was incubated at 42°C for 60 min, and the reaction was stopped by putting on ice. All compounds used for cDNA synthesis were obtained from Roche Molecular Biochemicals, Mannheim, Germany.

Primers were design to be complementary to the splice junction.

PCR amplification

A 210 bp fragment of *hCGβ* was amplified from cDNA using the following primers: sense 5'-GCAGGGGACGCACCAAGGA-3' (nucleotides 8-18, according to cDNA sequence, PubMed - AC: J00117) and antisense 5'-CACGCGGGTCATGGTGGG-3' (complementary to nucleotides 200-217).

A specific fragment of the β-actin gene was amplified from cDNA synthesized with universal oligo (dT)10 primer using RNA isolated from tissue from the negative control group. The reaction was carried out in order to check the quality of RNA in case of negative amplification of *hCGβ*.

A 604 bp fragment of ß-actin was amplified using the following primers: sense 5'-CATGTACGTTGCTATCCAGGC-3' (nucleotides 2057-2078, PubMed – AC: M10277) and antisense: 5'-CAGACAGCACTGCTGTGTTGGC-3' (nucleotides 2644-2661, PubMed – AC: M10277).

The amplification was performed in a reaction mixture containing: 1x Taq DNA polymerase buffer, 2.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.25 µM of each primer and 1 unit of Taq DNA polymerase, with a thermal profile as follows: 5 min at 95°C, 1 min at 95°C, 45 sec at a temperature specific for the primer set, 1 min at 72°C for 30 cycles. All compounds used for DNA synthesis were obtained from Bioline, London, UK. The amplified products (half of the reaction mixture) were electrophoresed on 1% agarose gel (FMC BioProducts, Rockland, Maine, USA).

Immunohistochemistry

Paraffin sections of the study tissue were used for immunohistochemical analysis. Antigens were retrieved by microwave activation in citrate buffer (10 mmol/l citrate, pH 6.0). Nonspecific protein binding was blocked in PBS solution containing 3% bovine serum albumin, and 0.1% Tween 20 and sections were incubated with commercial polyclonal antibodies - rabbit anti-human chorionic gonadotropine primary antibodies (DAKO A/S, Glostrup, Denmark) in the same solution for 60 minutes at 37°C in a humid chamber. The antibodies were used at a dilution of 1:200-1:600. After incubation, the slides were washed in PBS containing 0.1% Tween 20. The primary antibodies were detected using Cy3-conjugated sheep anti-rabbit IgG, diluted at 1:200 (Sigma-Aldrich, Saint Louis, Mi, USA). For negative controls, the primary antibodies were replaced with blocking solution. For localization of the secondary antibodies a Carl Zeiss LSM 510 confocal microscope was used.

Results

Tissue expression of mRNA hCGb

Ovarian cancer group. A specific region of beta-subunits of human chorionic gonadotropin was amplified from total RNA through reverse transcription followed by polymerase chain reaction (RT-PCR). Primers specific for human *hCGβ* were used and a 210 bp fragment of *hCGβ* was detected. In all 15 studies human tissue of an ovarian carcinoma specific fragment of *hCGβ* was present (Figure 1, lanes 2-4).

Control group. PCR with cDNA synthesized from total RNA isolated from human placentas, run as a positive control, showed the presence of hCGβ transcripts (Figures 1 and 2, lane 1).

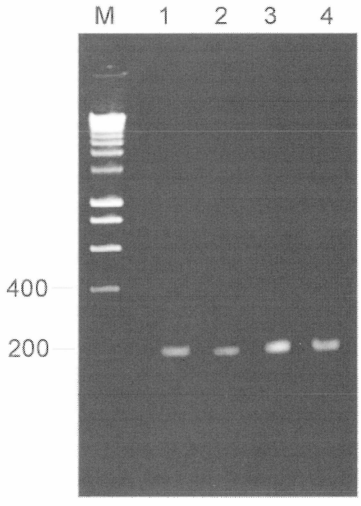


Figure 1. — RT-PCR analysis of *hCGβ* in ovarian cancer. Agarose gel resolution of products for *hCGβ* (210 bp) amplified from normal placenta - positive control - lane 1 and tumor tissue of ovarian carcinoma expressing *hCGβ* – lanes 2-4.

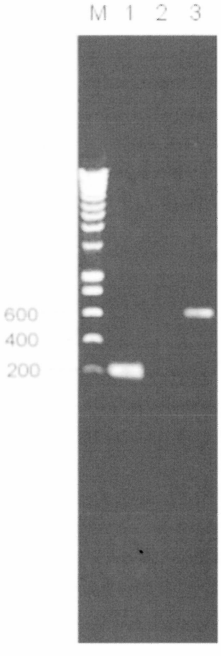


Figure 2. — RT-PCR analysis of hCGb in the negative control tissue. No product was observed when the tissue of women's genital tract lacking cancerous changes was tested, lane 2. In these cases *β-actin* product (604 bp) is shown to be a control of cDNA integrity - lane 3. Lane 1 - *hCGβ* (210 bp) product amplified from normal placenta.

ig. 3

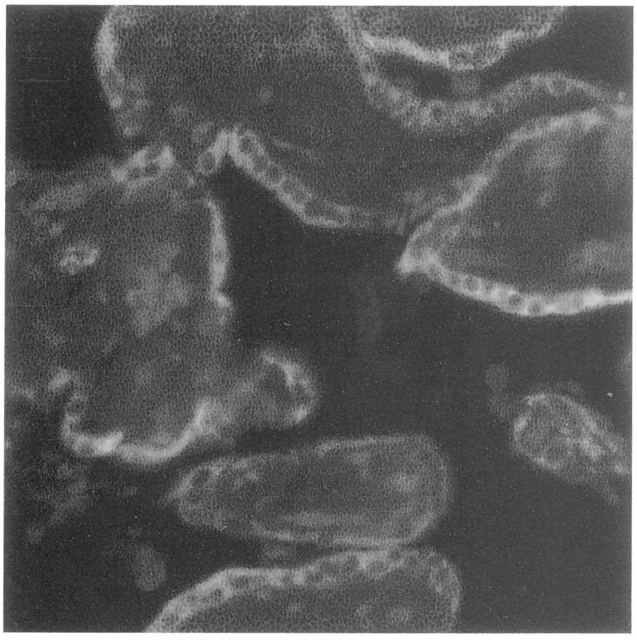
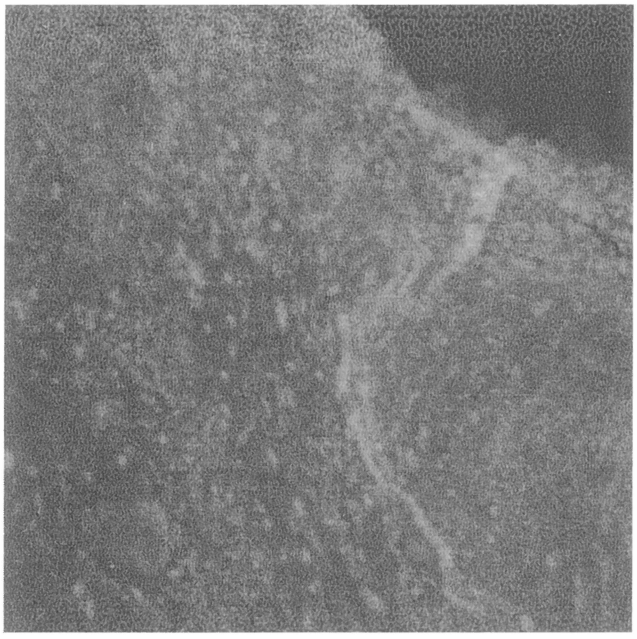


Fig. 4



g. 5

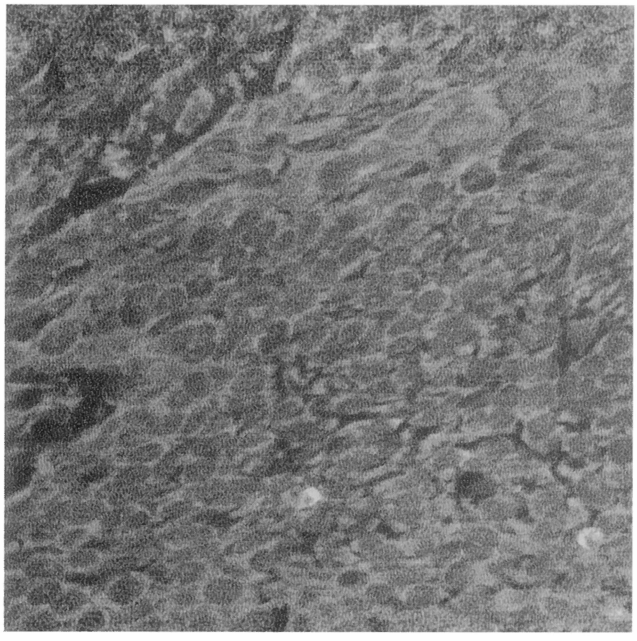


Fig. 6

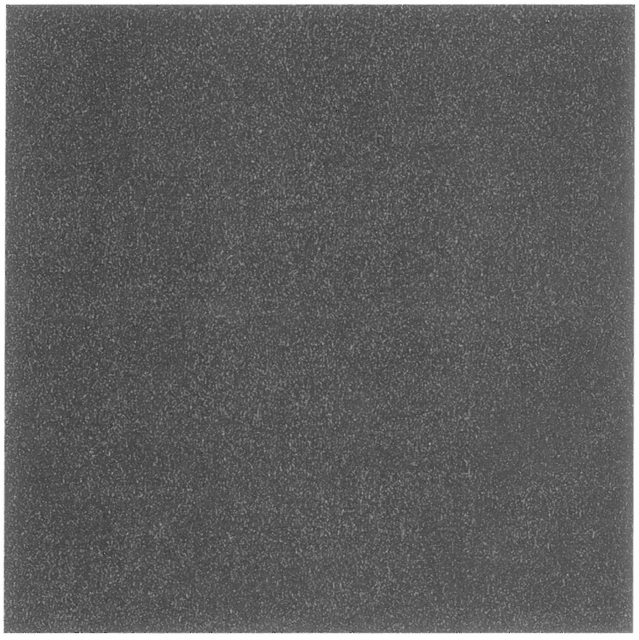


Figure 3. — Immunohistochemical analysis of hCGβ distribution in the placenta. Characteristic bright fluorescence corresponding to the protein presence visible in syncytiotrophoblasts (magnification x 630).
Figure 4. — Heterogeneous distribution of the hormone in ovarian carcinoma tissue (magnification x 400).
Figure 5. — Cluster organization of cancer cells expressing *hCGβ* (magnification x 630).
Figure 6. — Lack of the protein in normal endometrial epithelium (magnification x 100).

cDNA synthesized from total RNA, isolated from the women's genital tract with no cancerous changes, was run as a control and did not show the presence of *hCGβ* (Figure 2, lane 2). In these cases, the RT-PCR amplification of *β-actin* gene gave positive results. A 604 bp fragment of β-actin was detected in all study cases, showing the presence of intact RNA (Figure 2, lane 3).

The results of the immunohistochemical study performed on paraffin-embedded sections of tumor tissue showed the presence of beta subunits of human chorionic gonadotropin in all study cases in both the placenta (Figure 3) and cancerous tissue. The distribution of human chorionic gonadotropin in ovarian carcinoma was heterogeneous and characterised not all of the cells of the study tissue. The cells expressing hCGβ were usually organized in clusters characterized by uniform and homogeneous cytoplasmic staining (Figures 4 and 5). In the tissue lacking any cancerous changes hCGβ was not detected (Figure 6).

The choice of the control group allowed the determi-

nation that the expression of *hCGβ* is the property of tumor cells. The tissue, derived from the same operation materials lacked any cancerous changes, and when estimated by pathologic macroscopic examination, did not show any signs of *hCGβ* expression.

Discussion

The present study was undertaken to determine the expression and distribution of hCGβ in nontrophoblastic gynecological cancers. The findings indicate that both mRNA and protein products of the hCGβ gene are present in ovarian carcinoma tissue. This is, to our knowledge, the first report which directly confirms the production of hCGβ in ovarian tumors. At the same time we showed that noncancerous tissue of the same women's genital tract demonstrated no expression of the hormone. Thus, the present study shows that the expression of *hCGβ* is the characteristic feature of tumor tissue but its role in tumorigenesis is unknown. It was demonstrated that dimeric hCG forms the cystine knot structure [20, 21]. The structure of hCG resembles the structure of known growth factors, namely TGFβ (transforming growth factor), NGF (nerve growth factor) and PGDFβ (platelet derived growth factor), thus suggesting the promoting role of hCG in tumor growth [22-25].

The results of our study as well as other reports demonstrated that in addition to placenta and malignant trophoblastic disease, varieties of tumors of different origin secret hCG and especially its β-subunit [3-5]. The presence of hCG in any of its forms is treated as an *in vivo* phenotypic characteristic of human cancer cells [6]. Intact hCG appears to be the main form of hCG immunoreactivity, whereas the expression of the free β subunit – hCGβ – has been associated with the metastatic phenotype of cancer cells, but the biological mechanisms behind this association remain unclear [6, 8, 9]. We agree with Lundin *et al.*, who showed that hCGβ measured immunohistochemically in tumor tissue is an independent prognostic factor in colorectal cancer, however hCGβ expressed in tumor tissue and serum has prognostic significance independent of other clinicopathological variables [26]. It was also demonstrated that positive tumor staining does not always occur together with elevated serum levels, and the prognostic accuracy can be slightly increased by combining the results [27].

Our immunohistochemical study showed the distribution of cancer cells expressing hCGβ in ovarian tumors. The positively stained cells were present in all study cancer tissue samples, however in some cases only single cells expressing hCGβ were observed. The tissue without any cancerous changes did not show the presence of hCGβ.

Further studies are required to clarify whether hCGβ can be used when selecting patients for adjuvant therapy or intensive follow-up.

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