

## Localization of human chorionic gonadotropin beta subunit transcripts in ovarian cancer tissue

Ewa Nowak-Markwitz<sup>1</sup>, Anna Jankowska<sup>2</sup>, Anna Szczerba<sup>2</sup>, Mirosław Andrusiewicz<sup>2</sup> and Jerzy B. Warchol<sup>2</sup>

<sup>1</sup>Division of Obstetric and Gynecology and

<sup>2</sup>Department of Radiobiology and Cell Biology, University of Medical Sciences, Poznań, Poland

---

**Abstract:** Recent studies demonstrated that besides placenta and malignant trophoblastic tumors, hCG and especially its  $\beta$ -subunit is secreted by a varieties of tumors of different origin. The aim of the present investigation was to determine the expression pattern of human chorionic gonadotropin gene in ovarian cancer tissue. The study included 8 patients with epithelial ovarian carcinoma. The expression and distribution of *hCG $\beta$*  mRNA was assessed by *in situ* RT-PCR method. The semi-quantitative assessment was performed using computer image analysis. Transformation of the images into the pseudocolour scale showed a clear difference in fluorescence intensity among individual cancer cells. The intensity of ISRT-PCR products corresponding with expression level of *hCG $\beta$*  demonstrated that its production by individual cancer cells is different. In all studied specimens of the ovarian carcinoma tissue, cancer cells characterized by the presence of active *hCG $\beta$*  gene were found, whereas noncancerous tissue demonstrated lack of the gene expression. Thus, the study clearly shows that the expression of *hCG $\beta$*  is the feature of ovarian cancer tissue.

**Key words:** *hCG $\beta$*  - RT-PCR *in situ* - Pseudocolour images - Ovarian cancer

---

### Introduction

Human chorionic gonadotropin (hCG) is a sialoglycoprotein hormone composed of two noncovalently linked subunits -  $\alpha$  (hCG $\alpha$ ) and  $\beta$  (hCG $\beta$ ) [24]. Physiologically, hCG is produced by syncytiotrophoblastic cells of the placenta and secreted into blood, being present in urine of pregnant women and patients with trophoblastic diseases [3, 4]. The production of hCG, especially its  $\beta$ -subunit by patients with nontrophoblastic cancers has been reported by many authors and immunoreactive hCG/hCG $\beta$  has been detected in the blood of patients with a variety of tumors of different origin [3, 5, 8, 9, 11, 16, 20, 23, 25]. The presence of hCG in any of its forms is regarded as an *in vivo* phenotypic characteristic of human cancer cells [16].

The role of hCG in tumorigenesis is unknown but recent reports suggest that hCG $\beta$  can stimulate growth of cancer cells or inhibit the apoptosis and the elevated serum level of hCG $\beta$  correlates with higher aggressiveness of cancer and its resistance to the therapy [7, 15].

Intact hCG appears to be the main form of hCG immunoreactivity, whereas the expression of the free  $\beta$  subunit of hCG $\beta$  has been associated with metastatic phenotype of cancer cells, but the biological mechanisms behind this association remains unclear [8, 16, 23]. Although the serum immunoreactivity of hCG and hCG $\beta$  has been observed in patients with gynecological malignancies, there is almost no data of human chorionic gonadotropin expression in gynecological cancer tissue [12].

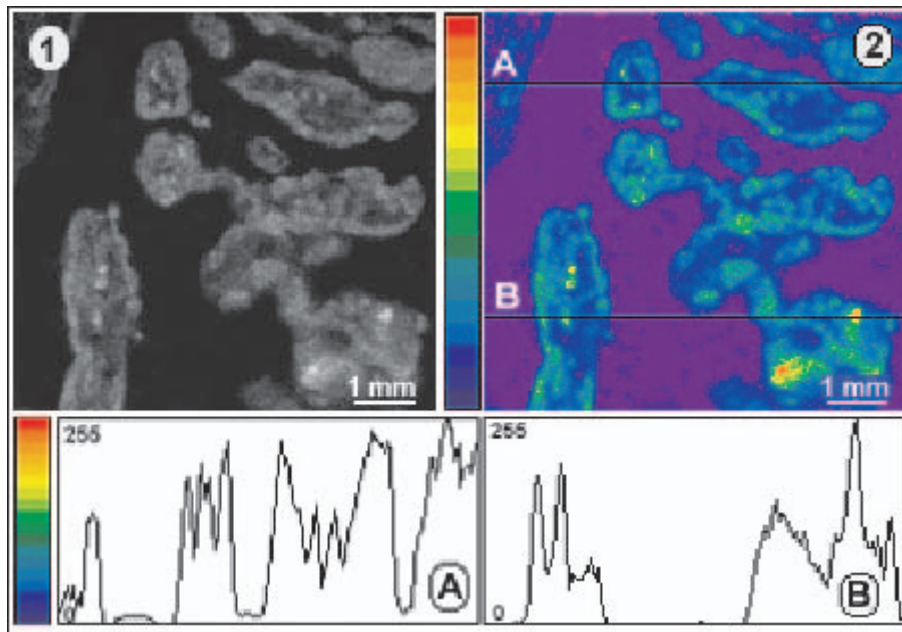
The aim of the present study was to analyze expression of human chorionic gonadotropin gene in epithelial ovarian cancer tissue.

### Materials and methods

**Tissue samples.** Surgical specimens of cancer tissue were obtained from 8 patients with epithelial ovarian carcinoma (median age 55 yrs, range 40-69 yrs), treated by surgery at the Department of Gynecologic Oncology, Poznań University of Medical Sciences in 2003. In all patients, histological confirmation of the cancer, including tumor grading, was obtained and the staging was performed according to the International Federation of Gynecology and Obstetric (FIGO). Histology groups were as follows: ovarian cancer of serous type (5 cases, tumor grading G1 - 1, G2 - 2, G3 - 2; FIGO IA - 1, IC - 1, IIC - 1, III - 2, IV - 1), ovarian cancer of mucinous type (1 case, tumor grading not determined, FIGO III), clear cell type (1 case, tumor

---

**Correspondence:** A. Jankowska, Dept. Radiobiology and Cell Biology, University of Medical Sciences, Święcickiego 6, 60-781 Poznań, Poland; e-mail: ajanko@amp.edu.pl



**Fig. 1.** The results of ISRT-PCR in ovarian cancer tissue. The cells containing *hCG* gene products are visible as bright spots. **Fig. 2.** The same image after transformation to pseudocolours. The cells containing *hCG* gene products are visible as yellow to red spots in cancer tissue. The weakly fluorescent green cells do not express *hCG*. **A, B.** The plots of the fluorescence intensity of the cells measured along lines A and B, respectively, shown in Fig. 2.

grading G2, FIGO III), and solid type (1 case, tumor grading G3, FIGO III).

The control samples obtained from the same patients consisted of surgically removed tissue which did not reveal any pathological changes after macroscopic and histopathological examinations: endometrium (2 samples) and the uterine cervix (1 sample) were collected. Two samples of placentas from term delivery served as a positive control.

The samples, collected at the operation were immediately fixed in 4% buffered formaldehyde, processed, embedded in paraffin and stored at 4°C. The patients were not treated by chemotherapy or radiotherapy prior to operation.

**RT PCR *in situ*.** In order to detect the *hCGβ* mRNA, the paraffin sections were prepared according to the procedure described by Bagasra and coworkers [2, 24]. Reverse transcriptase *in situ* was performed using Expand Reverse Transcriptase (Roche Molecular Biochemicals, Mannheim, Germany) with the primer specific for the *hCGβ* gene (reverse primer: 5'-GAGAAGCCTTTATTGTG-3', complementary to nucleotides 51595-51611, PubMed, AC: NG000019). A 210 bp fragment of cDNA of the *hCGβ* gene transcript was amplified by *in situ* PCR, using the following primers: sense 5'-GCAGGGGACGCACCAAGGA-3' (nucleotides 50499-50518 according to DNA sequence, PubMed, AC: NG000019) and antisense 5'-CACGCGGGTCATGGTGGG-3' (nucleotides: 51044-51296). The primers were designed to be complementary to the splice junction. Fluorescence labelled nucleotide Cy<sup>TM</sup>3-dUTP (Amersham Biosciences, Little Chalfont Buckinghamshire, UK) was used in order to identify the amplified product. *hCGβ* mRNA distribution was analysed using Carl Zeiss LSM 510 confocal microscope.

**Computer generation of pseudocolours.** The semi-quantitative analysis of *hCGβ* expression was based on computer image analysis. Images generated in .lsm true colour file format from Zeiss LSM image Browser Version 3,1,0,99 for Carl Zeiss Laser Scanning System LSM 510 (Carl Zeiss GmbH) were exported to Microsoft<sup>®</sup> Windows<sup>®</sup> Bitmap File Format and saved. Using Adobe Photoshop<sup>®</sup> 5.5 CEEA (Adobe Systems Inc.1989-1999), colour mode of the images was changed to greyscale format (8 bit per channel; 256 grey levels) and stored as Microsoft Windows<sup>®</sup> 8 Bitmap File Format. Then, using the Scion Image<sup>®</sup> (Release beta 4.0.2) for Windows

software (Scion Corporation<sup>®</sup> 2000), the greyscale was transformed into 32 colour table mode and stored as pseudocolour images.

## Results

The results of the *in situ* RT-PCR (ISRT-PCR) study performed on paraffin sections of tumour tissue demonstrated the presence mRNA for beta subunit of human chorionic gonadotropin in all cases, both in placenta (data not shown) and in cancerous tissue. The distribution of *hCGβ* mRNA in ovarian carcinoma was heterogeneous: not all cells of the studied tissue revealed the presence of the transcripts. The cells containing *hCGβ* mRNA were usually organized in clusters characterized by uniform and homogenous cytoplasmic staining. Computer analysis and the use of pseudocolour scale showed a clear difference in fluorescence intensity among individual cancer cells. The intensity of ISRT-PCR products corresponding with expression level of *hCGβ* demonstrated that its production by individual cancer cells is different (Figs. 1, 2). The fluorescence intensity measured after transformation into greyscale reached even maximal level for some cancer cells, whereas the other cells showed only weak or none fluorescence (Figs. A, B). In the control tissue from the same surgically removed material lacking the cancerous changes, the *hCGβ* mRNA was not detected (data not shown).

## Discussion

The present study was undertaken to determine the expression and distribution of *hCGβ* mRNA in nontrophoblastic gynecological cancers. The study was based

on ISRT-PCR technique which allows for amplification of the signal, thus is more sensitive than *in situ* hybridization [2, 13, 18, 24]. The findings of our research indicate that mRNA of *hCG* $\beta$  gene is present in ovarian carcinoma tissue. This is, to our knowledge, the first report, which directly revealed the production and distribution of *hCG* $\beta$  in ovarian tumors. At the same time we demonstrated that noncancerous tissue of the same women's genital tract did not demonstrate the expression of the gene. Thus, the present study shows that expression of *hCG* $\beta$  is characteristic feature of the tumor tissue but its role in tumorigenesis is unknown.

The result of our study as well as other reports demonstrated that besides placenta and malignant trophoblastic diseases, a variety of tumors of different origin secrete hCG and especially its  $\beta$ -subunit [3, 11, 20]. Recently, *hCG* $\beta$ -related low molecular weight material has been demonstrated in the urine of pregnant women and patients with trophoblastic and nontrophoblastic tumors [14, 17, 21]. This material was termed *hCG* $\beta$ -CF because it retains the *hCG* $\beta$  core conformation determinant recognized by *hCG* $\beta$  core antiserum but lacks the carboxy-terminal portion of *hCG* $\beta$  [1]. Currently,  $\beta$ -CF is receiving attention as a potential tumor marker since elevated levels of  $\beta$ -CF were frequently found in the urine of patients with nontrophoblastic tumors even if immunoreactive *hCG* $\beta$  could not be detected in the serum [6, 19, 26]. The study performed by Higashida *et al.* demonstrated the production of immunoreactive human chorionic gonadotropin  $\beta$ -subunit in case of one patient with ovarian malignant mixed mesodermal tumor [10].

Our study based on ISRT-PCR technique showed the distribution of cancer cells expressing the *hCG* $\beta$  in ovarian tumors. The positively stained cells were present in all studied cancer tissue samples, however, in some cases only single cells expressing *hCG* $\beta$  were observed. The tissue lacking the cancerous changes did not show the presence of *hCG* $\beta$  mRNA. Computer analysis and use of pseudocolour scale showed the difference in fluorescence intensity among individual cancer cells of the analysed tissue. The intensity of ISRT-PCR product fluorescence corresponding with the expression level of *hCG* $\beta$  showed that its production by individual cells is different. Further investigations should allow to determine the type of cancer cells expressing *hCG* $\beta$  and to find out whether and when the cells possess the ability to produce *hCG* $\beta$  in preneoplastic lesions.

**Acknowledgments:** We thank Prof. Helena Kędzia for help in pathological examination of the tissue. The research was supported in the part by National Committee for Scientific Research (KBN) grant no. 3PO5E 162 22.

## References

- [ 1] Amr S, Wehmann RE, Birken S, Canfield RE, Nisula BC (1983) Characterization of a carboxyterminal peptide fragment of the human chorionic gonadotropin  $\beta$ -subunit excreted in the urine of a woman with choriocarcinoma. *J Clin Invest* 71: 329-339
- [ 2] Bagasra O, Seshamma T, Pomarantz R, Hansen R (1995) *Current Protocols in Molecular Biology*; Vol 2, Suppl 31: 14.8.1-14.8.232
- [ 3] Bagshawe KD (1992) Choriocarcinoma. A model of tumor markers. *Acta Oncol* 31: 99-106
- [ 4] Braunstein GD, Karow WG, Gentry WC, Rasor J, Wade ME (1978) First-trimester chorionic gonadotropin measurements as an aid in the diagnosis of early pregnancy disorders. *Am J Obstet Gynecol* 131: 25-32
- [ 5] Carpelan-Holmström M, Haglund C, Lundin J, Alftan H, Stenman U-H, Roberts PJ (1996) Independent prognostic value of preoperative serum markers CA-125, specific tissue polypeptide antigen and human chorionic gonadotropin  $\beta$ , but not carcinoembryonic antigen or tissue polypeptide antigen in colorectal cancer. *Br J Cancer* 74: 925-929
- [ 6] Cole LA, Wang YX, Elliott M, Latif M, Chambers JT, Chambers SK, Schwartz PE (1988) Urinary human chorionic gonadotropin free  $\beta$ -subunit and  $\beta$ -core fragment: a new marker of gynecological cancers. *Cancer Res* 48: 1356-60
- [ 7] Gillott DJ, Iles RK, Chard T (1996) The effect of  $\beta$ hCG on the *in vitro* growth of bladder cancer cells. *Br J Cancer* 73: 323-326
- [ 8] Grossman M, Hoermann R, Gocze PM, Ott M, Berger P, Mann K (1995) Measurement of human chorionic gonadotropin-related immunoreactivity in serum, ascites and tumor cysts of patients with gynecologic malignancies. *Eur J Clin Invest* 25: 867-873
- [ 9] Grossman M, Trautmann ME, Poertl S, Hoermann R, Berger P, Arnold R, Mann K (1994)  $\alpha$ -subunit and human chorionic gonadotropin- $\beta$  immunoreactivity in patients with malignant endocrine gastroenteropancreatic tumors. *Eur J Clin Invest* 24: 131-136
- [10] Higashida T, Koizumi T, Yamaguchi S, Ichimura T, Hasegawa K, Nishimura R (2001) Ovarian malignant mixed mesodermal tumor producing the free form of the  $\beta$ -subunit of human chorionic gonadotropin. *Int J Clin Oncol* 6: 97-100
- [11] Husa RO (1981) Human chorionic gonadotropin, a clinical marker: review of its biosynthesis. *Ligand Rev* 3: 6-44
- [12] Ind T, Iles RK, Shepherd J, Chard T (1997) Serum concentrations of cancer antigen 125, placental alkaline phosphatase, cancer-associated serum antigen and free  $\beta$  human chorionic gonadotropin as prognostic markers for epithelial ovarian cancer. *Br J Obstet Gynecol* 104: 1024-1029
- [13] Kasprzak A, Zabel M, Wysocki J, Surdyk-Zasada J, Filipiak B (2002) Expression of mRNA for cytokines (TNF- $\alpha$  and IL-1 $\alpha$ ) in human cytomegalovirus (HCMV) and hepatitis B virus (HBV) infections. *Folia Histochem Cytobiol* 40: 63-68
- [14] Kato Y, Braunstein GD (1988)  $\beta$ -core fragment is a major form of immunoreactive urinary chorionic gonadotropin in human pregnancy. *J Clin Endocrinol Metab* 66: 1197-1201
- [15] Lunardi-Iskander Y, Bryant JL, Zeman RA, Lam VH, Samaniego F, Besnier JM, Hermans P, Thierry AR, Gill P, Gallo RC (1995) Tumorigenesis and metastasis of neoplastic Kaposi's sarcoma cell line in immunodeficient mice blocked by a human pregnancy hormone. *Nature* 375: 64-68
- [16] McLoughlin J, Pepera T, Bridger J, Williams G (1991) Serum and urinary levels of  $\beta$  chorionic gonadotropin in patients with transitional cell carcinoma. *Br J Cancer* 63: 822-824
- [17] Nishimura R, Kitajima T, Hasegawa K, Takeuchi K, Mochizuki M (1989) Molecular forms of human chorionic gonadotropin in choriocarcinoma serum and urine. *J Cancer Res* 80: 968-74

- [18] Nowicki M, Miskowiak B, Ostalska-Nowicka D (2003) Detection of substance P and its mRNA in human blast cells in childhood lymphoblastic leukaemia using immunocytochemistry and *in situ* hybridisation. *Folia Histochem Cytobiol* 41: 33-36
- [19] O'Connor JF, Schlatterer JP, Birken S, Krichevsky A, Armstrong EG, McMahon D, Canfield RE (1988) Development of highly sensitive immunoassays to measure human chorionic gonadotropin, its beta-subunit, and beta core fragment in the urine: application to malignancies. *Cancer Res* 48: 1361-1366
- [20] Ozturk M (1991) Human chorionic gonadotropin, its free subunits and gestational trophoblastic disease. *J Reprod Med* 36: 21-26
- [21] Papapetrou PD, Nicopoulou SC (1986) The origin of a human chorionic gonadotropin beta-subunit-core fragment excreted in the urine of patients with cancer. *Acta Endocrinol (Copenh)* 112: 415-422
- [22] Pierce JG, Parson TF (1981) Glycoprotein hormones: structure and function. *Annu Rev Biochem* 50: 465-495
- [23] Szturmowicz M, Wiatr E, Sakowicz A, Słodkowska J, Roszkowski K, Filipecki S, Rowinska-Zakrzewska E (1995) The role of human chorionic gonadotropin  $\beta$ -subunit elevation in small-cell lung cancer patients. *Can Res Clin Oncol* 121: 309-312
- [24] Warchol JB, Augustyniak S, Stecewicz D, Jankowska A (2001) Detection of DAZ mRNA distribution in human testis using reverse transcription *in situ* PCR technique (RT-ISPCR). *Folia Histochem Cytobiol* 39: 117-118
- [25] Webb A, Scott-Mackie P, Cunningham D, Norman A, Andreyev J, O'Brien M, Bensted J (1996) The prognostic value of serum and immunohistochemical tumor markers in advanced gastric cancer. *Eur J Cancer* 32: 63-68
- [26] Yoshimura M, Nishimura R, Murotani A, Miyamoto Y, Nakagawa T, Hasegawa K, Koizumi T, Shii K, Baba S, Tsubota N (1994) Assessment of urinary beta-core fragment of human chorionic gonadotropin as a new tumor marker of lung cancer. *Cancer* 73: 2745-2752

*Accepted January 13, 2004*