Placental mRNA in Maternal Plasma and Its Clinical Application to the Evaluation of Placental Status in a Pregnant Woman with Placenta Previa-Percreta

To the Editor:

Fetal and/or placental mRNA in maternal plasma has been detected during pregnancy, and such mRNA tends to be stable against degradation (1). A quantitative study of plasma mRNA for γ-globin and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) showed significantly higher concentrations in pregnant women than in nonpregnant women (2). These findings suggest that the quantitative analysis of placental mRNA in maternal plasma may be a useful method to monitor placental status.

In this study, we measured placental mRNA in maternal plasma to evaluate residual placenta in a pregnant woman with placenta previa-percreta (PPP) and bladder invasion that was diagnosed by both magnetic resonance imaging and pathology examination. Although a supravaginal hysterectomy just after the cesarean section (the first surgery) was done at 37 weeks of gestation, a 16-cm placental mass close to the internal os of the uterus could not be removed. Therapy with methotrexate (MTX) was therefore initiated on days 1–4, days 14–17, and days 33–36 after the first surgery (on day 0) to aid resorption of the placental residue, and a second surgery was performed to complete the hysterectomy on day 75. The study protocol was approved by the Committee for the Ethical Issues on Human Genome and Gene Analysis in Nagasaki University, and written informed consent was obtained. Data regarding plasma mRNA concentrations did not influence clinical management.

Blood samples (10 mL) were collected at intervals and before and after the surgeries. Plasma mRNA was extracted as described by Ng et al. (1). Human chorionic gonadotropin-β (hCG-β) and human placental lactogen (hPL) were selected as representative placental mRNAs, and GAPDH mRNA was measured as a

DOI: 10.1373/clinchem.2005.048231

Natalia Ferré1 Jordi Camps2* Judit Marsillach1 Bharti Mackness2 Mike Mackness1 Blai Coll1 Mónica Tous1 Jorge Joven1

1 Centre de Recerca Biomèdica Hospital Universitari de Sant Joan Institut de Recerca en Ciències de la Salut Reus, Catalunya, Spain

2 University Department of Medicine Manchester Royal Infirmary Manchester, United Kingdom

*Address correspondence to this author at: Centre de Recerca Biomèdica, Hospital Universitari de Sant Joan, Institut de Recerca en Ciències de la Salut, C/. Sant Joan s/n, 43201-Reus, Catalunya, Spain. Fax 34-977-312569; e-mail j camps@grupsgesssa.com.
housekeeping gene. A one-step real-time reverse transcription (RT)-PCR assay (EZ rTh RNA PCR Kit; Applied Biosystems) was performed on an Applied Biosystem 7900T Sequence Detector (Perkin-Elmer) to measure the mRNA concentration in maternal plasma. Primer sets and TaqMan probes for the 3 genes selected were prepared as described previously (1). Calibration curves for the quantification of each mRNA were prepared by assaying serial dilutions of HPLC-purified single-strand synthetic DNA oligonucleotides from each PCR amplicon ($R^2 = 0.99$; 95% confidence interval for the slope of the calibration curve for the real-time RT-PCR, 2.9 to 3.3). The absolute concentration of each mRNA was expressed as copies/mL of maternal plasma, based on the formula described by Farina et al. (3). Calibration curves for hCG-$\beta$ and hPL mRNA ranged from $1 \times 10^7$ to $1 \times 10^9$ copies/mL, and the curve for GAPDH mRNA from $1 \times 10^{10}$ to $1 \times 10^{14}$ copies/mL. Each sample was analyzed in triplicate with thermal cycling as described previously (1).

The hCG-$\beta$ mRNA showed a decreasing tendency similar to the pattern for hCG protein concentrations measured by an IRMA, but exhibited a transient increase to $1.90 \times 10^3$ copies/mL on day 12 after the first MTX therapy and then decreased again to $<10$ copies/mL after the second surgery (Fig. 1A). Interestingly, plasma hPL mRNA concentrations also showed a decreasing tendency but increased again after the second MTX therapy from $7.88 \times 10^2$ copies/mL on day 15 to $2.68 \times 10^3$ copies/mL on day 19, whereas the hCG-$\beta$ mRNA concentration decreased (Fig. 1B). This discrepancy may be attributable to different expression patterns of the hPL and hCG-$\beta$ genes in the placental cell. hCG-$\beta$ mRNA is produced primarily in proliferating cytotrophoblasts and to a lesser extent in mature syncytiotrophoblasts, whereas the hPL gene is expressed mainly in the mature syncytiotrophoblast (4). Because MTX strongly inhibits DNA synthesis, the hCG-$\beta$ mRNA concentration may reflect the MTX-induced apoptotic activity in dividing cytotrophoblasts directly and the hPL mRNA concentration may reflect the resorption of syncytiotrophoblasts. Because the placenta at term has many mature syncytiotrophoblasts, combined monitoring of hCG-$\beta$ and hPL mRNA concentrations in maternal plasma can be used for evaluation of chemotherapeutic efficacy of MTX for PPP.

The GAPDH mRNA concentrations in plasma also increased after both surgeries and after the first MTX therapy (Fig. 1C). Thus, cell/tissue damage by both surgery and...
chemotherapy may be associated with increased concentrations of GAPDH mRNA. Because circulating GAPDH mRNA in cancer patients was thought to originate in apoptotic cancer cells (2, 5), the increased concentrations of GAPDH mRNA detected may be caused by apoptosis in the placental residue in our case. We conclude that real-time quantitative RT-PCR is a sensitive method to monitor changing mRNA concentrations resulting from apoptotic effects in the placenta and to evaluate invading conditions of the trophoblastic villus.

We thank Dr. Joseph Wagstaff for help and valuable advice. This work was supported in part by Grants-in-Aid for Scientific Research (15591761, 16591670, and 13854024) from the Ministry of Education, Sports, Culture, Science and Technology of Japan (to H.M., K.M., and N.N.) and CREST from Japan Science and Technology Agency (JST; to N.N.). H. Masuzaki and K. Miura contributed equally to this work.

References


**Hideaki Masuzaki**

**Kiyonori Miura**

**Koh-ichiro Yoshiura**

**Kentaro Yamashita**

**Shoko Miura**

**Shuichiro Yoshimura**

**Daisuke Nakayama**

**Christophe K. Mapendano**

**Norio Niikawa**

**Tadayuki Ishimaru**

**Departments of**

1. Obstetrics and Gynecology and 2. Human Genetics

**Nagasaki University Graduate School of Biomedical Sciences**

**Nagasaki, Japan**

3. CREST

**Japan Science and Technology Agency**

**Kawaguchi, Japan**

*Address correspondence to this author: Department of Obstetrics and Gynecology, Nagasaki University Graduate School of Biomedical Sciences, Saka-moto 1-7-1, Nagasaki 852-8501, Japan. Fax 81-95-849-7365; e-mail kiyonori@net.nagasaki-u.ac.jp.*

DOI: 10.1373/clinchem.2005.047803