Urinary Cyclic GMP after Treatment of Gynecological Cancer. A Prognostic Marker of Clinical Outcome

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Abstract. *Background: The search for biological markers to predict malignant disease and its recurrence, or to monitor the effectiveness of treatment is a continuous process in medicine. Several years ago, urinary excretion of cGMP in urine was found to be a sensitive predictor in the follow-up of ovarian cancer and of monitoring treatment of cancer of the uterine cervix. Patients and Methods: In the present study, 27 patients with gynecological cancer, including cancer of the uterine cervix (n=13), cancer of the uterine corpus (n=8) and cancer of the ovaries (n=6), were monitored for 10 years. Blood and urinary samples were taken before primary treatment (baseline sample) and three months thereafter (three-month sample). The serum levels of CEA, CA-125 and PIIINP and urine excretion of cGMP and cAMP were determined. Creatinine levels in serum and urine were employed to determine renal clearance. Results: After 10 years' observation of women with cancer of the uterine cervix, seven patients showed no relapse and cGMP levels in baseline samples and three-month samples were 36.8±4.1 and 24.9±4.4 nmol cGMP/μmol creatinine (mean±SEM, p<0.01), respectively. The levels in patients (n=6) with relapse after 10 years' observation were 32.8±4.0 (baseline sample) and 43.5±4.2 (three-month sample) nmol cGMP/μmol creatinine (mean±SEM, p<0.02). Among the patients treated for cancer of the uterine corpus (n=9), none showed recurrent disease within the observation period of 10 years. The cGMP levels fell from 37.9±6.3 (baseline sample) to 22.3±2.3 (three-month sample) nmol cGMP/μmol creatinine (p<0.005). In the patients with ovarian cancer (n=6), 4 patients relapsed during the observation period of 10 years. In these women the cGMP levels increased from 34.5±2.7 (baseline sample) to 46.3±4.7 nmol cGMP/μmol creatinine whilst in both patients without relapse the levels decreased from 31.8 (range: 26.5-37.1) to 27.3 (range: 25.7-28.8) nmol cGMP/μmol creatinine, respectively. The changes in levels of cAMP, CEA, CA-125 and PIINP did not show statistically significant differences. Early changes in cGMP levels appear to predict long-term prognosis in gynecological cancers.*

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Key Words: Tumor marker, gynecological cancer, cGMP, prognosis.

Two decades ago urine cGMP was proposed as a tumor marker in cancer patients, including ovarian cancer (1). In a follow-up study with monitoring of ovarian cancer during chemotherapy, the levels of cGMP reflected response to therapy, but also predicted a recurrence of the tumor growth before any clinical signs (2). Later, the use of urine cGMP was excluded as a marker for screening purposes. However, after surgery and before administration of cytotoxic drugs, urine cGMP levels appeared to be an independent prognostic variable with respect to survival (3). In a follow-up study on patients with epithelial ovarian cancer, serial measurements of urine cGMP showed that 64% had a pre-chemotherapy cGMP level above the range for controls (4). In these patients, a 96% correlation of tumor regression witrh a fall in cGMP levels existed, and a 75% correlation of tumor progression with an increase in cGMP levels. Marker-positive patients also demonstrated a rise in marker before clinical recognition of disease progression in 55%, whereas only 11% of marker-negative patients showed such an elevation. Patients with evidence of static disease had normal and stable marker levels. Finally, the usefulness of urine cGMP as a cancer marker in ovarian cancer was studied in two independent centers with serial specimens from patients monitored up to 2 years after the start of chemotherapy (5). The urine cGMP levels were significantly higher in those patients with poorly differentiated tumors. If the cGMP levels were elevated during therapy, there was an 80-90% probability that the patient was a non-responder. Using serial measurements, it was possible to predict the recurrence of the tumor in 64% of the patients prior to any other clinical signs (5).

In patients (n=15) with uterine cervical cancer, urine cGMP concentrations were elevated (6) and in a study wherein the patients (n=12) were their own control with urine sampling before and after treatment (7), the levels fell significantly in patients with response to therapy, but remained high in non-responders or in patients with relapse. *In vitro* studies wherein the growing cells, derived from cancer of the human uterine cervix, mimicked the expanding tumor bulk *in vivo* showed a cell density-dependent increase of extracellular cGMP levels (8, 9).

For many years CA-125 has been the marker of choice for ovarian cancer, but more recently, additional biomarkers have been employed (10). Amino terminal propeptide of type III procollagen (PIIINP) is a marker which has been shown to have prognostic value in several gynecological malignancies, especially in ovarian cancer (11). CEA is mostly positive in gastrointestinal cancers, but has proven to be of some prognostic importance for cervical carcinomas (12). Tumor markers with optimal sensitivity and specificity are still lacking in diagnostics of gynecological cancer. In the present study we investigated the changes in urine cGMP and cAMP levels and in serum of other markers (CA-125, CEA, PIIINP) before and 3 months after primary therapy, and the patients were observed for 10 years in order to detect any relapse.

Table I. *Urine cGMP and cAMPin patients (n=13) with cancer of the uterine cervix before (baseline sample=sample 1) and three months after primary treatment (three month sample=sample 2). Urine cyclic nucleotides (nmol/l) were related to urine creatinine (μmol/l). The changes in cyclic nucleotide levels are related to the clinical diagnosis three months and 10 years after primary treatment.The levels are presented as the mean value±SEM.*

cGMP (nmol/μmol)	No relapse	Relapse
3 month follow-up	(n=9)	(n=4) *
Sample 1	33.8±3.7	37.5±4.2
Sample 2	27.3±3.8 ($p>0.05^b$)	47.4±5.1
10 year follow-up	(n=7)	(n=6)
Sample 1	36.8±4.1	32.8±4.0 ($p>0.05^a$)
Sample 2	24.9±4.4	43.5±4.2
	($p<0.01^b$)	($p<0.005^a$, $p<0.02^b$)
cAMP (nmol/μmol)	**No relapse**	**Relapse**
3 month follow-up	(n=9)	(n=4)*
Sample 1	16.8±3.0	23.5±3.6
Sample 2	18.0±3.1 ($p>0.05^b$)	23.2±4.3
10 year follow-up	(n=7)	(n=6)
Sample 1	16.8±3.6	21.3±3.2 ($p>0.05^a$)
Sample 2	17.3±3.3	22.4±3.8
	($p>0.05^b$)	($p>0.05^a$, $p>0.05^b$)

*Number in the relapse group after three months' observation considered too low for statistical analysis. [a]No relapse *versus* relapse; [b]Sample 1 *versus* sample 2.

Patients and Methods

Patients. Women with gynecological cancers were included when admitted to the University Hospital of Tromso for primary treatment. The group of patients with squamous cell carcinoma of the uterine cervix comprised 13 patients with a mean and median age of 50.5 and 54 years, respectively (range: 35-68 years). Staging according to FIGO showed four patients in stage I, six patients in stage II and three patients in stage III with grade II (n=12) or grade III (n=1). Relapse was diagnosed in four patients after three months and additionally three patients after 10 years. The patients (n=8) with cancer of the uterine corpus had a mean and median age of 59.2 and 61 years, respectively (range 43-73 years). Staging showed FIGO stage I (n=6), stage II (n=2) and stage III (n=1), grade I (n=4), grade II (n=4) and grade III (n=1). No recurrent disease was diagnosed within the observation period of 10 years. The patients (n=6) with ovarian cancer were characterized by a mean and median age of 65.2 and 65 years, respectively (range 59-74 years). Staging according to FIGO showed three patients in stage I, three patients in stage III and all (n=6) with grade II. After three months, three patients showed relapse and after 10 years additionally one patient showed recurrent disease. All the participants gave their informed consent. The patients were included in a control program of follow-up by an experienced gynecologist at the local hospital or at the University Hospital.

Blood sampling. The blood samples were obtained as a part of the routine investigation, prior to primary treatment (baseline sample=sample 1) and three months (three month sample=sample 2) thereafter.

Urine collection. Urine was collected for 24 h to determine creatinine clearance and levels of cyclic nucleotides. The urinary samples were obtained prior to primary treatment (baseline sample=sample 1) and 3 months (three month sample=sample 2) thereafter. The specimen was immediately frozen and stored at –22˚C until analysis.

Analysis of cyclic nucleotides. The concentrations of cGMP and cAMP were determined in duplicate by radioimmunoassay RIA (13). The urines were thawed, mixed with trichloroacetic acid to achieve the final concentration of 0.5% (v/v) and then neutralized with $CaCO_3$. Antiserum against cGMP, [^{3}H]-cGMP and non-radioactive cGMP, [^{3}H]-cAMP were purchased from Amersham International (Buckinghamshire, UK). Non-radioactive cAMP was obtained from Sigma Chemical Co (St. Louis, MO, USA). Antiserum against cAMP was raised in our own laboratory and tested with affinity and specificity as described elsewhere (14). The 24-h cGMP excretion was related to that of creatinine in order to correct for dilution of urine and any changes in renal function.

Analysis of CEA, CA-125 and PIIINP in serum. The assays were performed according the manufacturer's recommendations. The levels of CEA and CA-125 were determined with chemiluminescent immunoassays (Bayer, Lerverkusen, Germany) and PIINP levels were determined with RIA kits (Orion Diagnostics, Turku, Finland).

Statistics. Statistical analysis was performed using GraphPad InStat ver. 5.1 (GraphPad Software, San Diego, CA, USA). Descriptive statistics are presented as mean±SEM if not stated otherwise.

Table II. *CEA, CA-125 and PIIINP in serum of patients (n=13) with cancer of the uterine cervix, before (baseline sample=sample 1) and three months after primary treatment (three month sample=sample 2). The changes in levels are related to the clinical diagnosis three months and 10 years after primary treatment. The levels are presented as the mean value±SEM.*

CEA (ng/ml)	No relapse	Relapse
3 month follow-up	(n=9)	(n=4)*
Sample 1	2.7±0.4	2.3±0.4
Sample 2	1.8±0.4 ($p<0.02^b$)	1.7±0.2
10 year follow-up	(n=7)	(n=6)
Sample 1	3.0±0.4	2.0±0.3 ($p>0.05^a$)
Sample 2	1.9±0.5 ($p<0.05^b$)	1.6±0.2 ($p>0.05^a$, $p<0.05^b$)
CA-125 (units/ml)	**No relapse**	**Relapse**
3 month follow-up	(n=9)	(n=4)*
Sample 1	17.8±4.8	24.3±5.3
Sample 2	18.7±6.9 ($p>0.05^b$)	30.5±11.8
10 year follow-up	(n=7)	(n=6)
Sample 1	24.3±5.3	30.5±11.8 ($p>0.05^a$)
Sample 2	17.3±8.5 ($p>0.05^b$)	28.2±8.3 ($p>0.05^a$, $p>0.05^b$)
PIINP (ng/ml)	**No relapse**	**Relapse**
3 month follow-up	(n=9)	(n=4) *
Sample 1	2.5±0.3	4.2±1.1
Sample 2	2.5±0.2 ($p>0.05^b$)	4.5±1.0
10 year follow-up	(n=7)	(n=6)
Sample 1	2.7±0.4	3.4±0.9 ($p>0.05^a$)
Sample 2	2.3±0.3 ($p>0.05^b$)	3.9±0.8 ($p>0.05^a$, $p>0.05^b$)

*Number in the relapse group after three months' observation considered too low for statistical analysis. [a]No relapse *versus* relapse; [b]Sample 1 *versus* sample 2.

Table III. *Urine cGMP and cAMPin patients (n=6) with ovarian cancer before (baseline sample=sample 1) and three months after primary treatment (three month sample=sample 2). Urine cyclic nucleotides (nmol/l) were related to urine creatinine (μmol/l). The changes in cyclic nucleotide levels are related to the clinical diagnosis three months and 10 years after primary treatment. The levels are presented as the mean value (range).*

cGMP (nmol/μmol)	No relapse	Relapse
3 month follow-up	(n=3)*	(n=3)*
Sample 1	31.4 (26.5-37.1)	35.8 (30.6-41.9)
Sample 2	35.8 (25.7-53.0)	44.1 (36.7-55.5)
10 year follow-up	(n=2)*	(n=4)*
Sample 1	31.8 (26.5-37.1)	34.5 (30.6-41.9)
Sample 2	27.3 (25.7-28.8)	46.3 (36.7-55.5)
cAMP (nmol/μmol)	**No relapse**	**Relapse**
3 month follow-up	(n=3)*	(n=3) *
Sample 1	12.8 (10.3-17.5)	16.5 (8.4-31.0)
Sample 2	14.9 (5.4-21.1)	18.6 (13.0-25.6)
10 year follow-up	(n=2)*	(n=4)*
Sample 1	14.2 (10.8-17.5)	15.0 (8.4-31.0)
Sample 2	19.7 (18.3-21.1)	15.3 (5.4-25.6)

*Numbers considered too low for statistical analysis.

Results

Cancer of the uterine cervix. Clinical status revealed that four of the patients showed relapse after three months while two more patients had relapsed 10 years after primary treatment. Table I shows the values of urinary cGMP excretion. In each of the four patients with relapse after three months, cGMP excretion showed an increase. When the observation time was extended to 10 years, no significant difference in cGMP levels was observed before treatment, whereas significantly lower levels were seen in patients without relapse in post-treatment samples (Table I). In contrast, there were no changes in the excretion of cAMP before and after treatment or between the groups (Table I). With respect to relapse status after 10 years, the CEA levels were significantly lower post-treatment in both groups, but without any statistical differences between them (Table II). Neither the levels of CA-125 nor PIIINP showed any differences (Table II).

Cancer of the uterine corpus. None of these patients showed any recurrent malignant disease after three months or 10 years. The urine levels of cGMP levels fell from 37.9±6.3 to 22.3±2.3 nmol cGMP/μmol creatinine ($p<0.005$), whereas the cAMP levels were unchanged at 16.6±4.0 and 15.6±3.5 nmol cAMP/ μmol creatinine ($p>0.05$), respectively. No significant differences were obtained for CEA (1.1±0.2 ng/ml and 1.3±0.1 ng/ml, $p>0.05$), CA-125 (28.4±5.2 units/ml and 24.4±8.1 units/ml, $p>0.05$) and PIIINP (3.7±0.5 ng/ml and 4.2±0.4 ng/ml, $p>0.05$).

Ovarian cancer. After three months' observation, three patients showed residual disease/relapse. After 10 years' observation, one more patient had verified relapse. In this group (n=4) the cGMP levels were higher post-treatment for all patients (Table III), whereas the levels were lower in the two patients without relapse. In the two patients without relapse after 10 years, the cAMP values levels were higher post-treatment, whereas the four patients with relapse showed no consistent changes in cAMP levels (Table III).

When the CEA values were related to clinical outcome after 10 years, the level increased from baseline to 3 months in patients without relapse, whereas the levels, being much higher before treatment, were unaltered in the four patients with relapse (Table IV). The levels of CA-125 fell after treatment in both groups, but apparently much more in the patients without relapse (Table IV). The levels of PIINP

Table IV. *CEA, CA-125 and PIIINP in serum of patients (n=6) with ovarian cancer, before (baseline sample=sample 1) and three months after primary treatment (three month sample=sample 2). The changes in levels are related to the clinical diagnosis three months and 10 years after primary treatment. The levels are presented as mean value (range).*

CEA (ng/ml)	No relapse	Relapse
3 month follow-up	(n=3)*	(n=3)*
Sample 1	1.7 (0.7-3.1)	2.3 (0.8-4.8)
Sample 2	2.5 (0.9-3.9)	1.9 (0.8-3.5)
10 year follow-up	(n=2)*	(n=4)*
Sample 1	1.1 (0.7-1.4)	2.5 (0.8-4.8)
Sample 2	1.8 (0.9-2.6)	2.4 (0.8-3.9)
CA-125 (units/ml)	No relapse	Relapse
3 month follow-up	(n=3)*	(n=3)*
Sample 1	182 (14-490)	110 (24-200)
Sample 2	63 (8-140)	55 (25-95)
10 year follow-up	(n=2)*	(n=4)*
Sample 1	250 (14-490)	94 (24-200)
Sample 2	75 (8-140)	51 (25-95)
PIINP (ng/ml)	No relapse	Relapse
3 month follow-up	(n=3)*	(n=3)*
Sample 1	3.5 (2.7-4.7)	11.4 (5.3-15.1)
Sample 2	3.7 (3.2-4.3)	7.4 (7.0-19.9)
10 year follow-up	(n=2)*	(n=4)*
Sample 1	3.0 (2.7-3.2)	9.7 (4.7-15.1)
Sample 2	3.8 (3.2-4.3)	9.4 (3.6-19.9)

*Number considered too low for statistical analysis.

increased in both patients without relapse after an observation period of 10 years. In the group with relapse, no consistent pattern was seen.

Discussion

Cyclic GMP as a tumor marker has been thoroughly studied in two main groups of malignancies: gynecological cancers (1-7) and leukemias (15-18). Cellular cGMP efflux is a general mechanism for cellular elimination of this signal molecule (19), together with hydrolysis by cyclic nucleotide phosphodiesterases (20). Member 5 of the superfamily of multidrug resistance proteins (MRP) has been shown to be involved the transport of cGMP (21-23). However, the contribution from MRP4 has not been settled (23-27). There is no general agreement on whether these proteins are high affinity or low affinity transporters for cGMP (21, 23, 25-27). With reference to the increased cGMP levels observed in leukemia (15-18), it is intriguing that expression of MRP5, but not MRP4 is correlated to prognosis of acute leukemia (28).

In the present study of gynecological cancers, urine and serum samples were obtained before (baseline sample) and three months (three month sample) after primary treatment to elucidate the possibility that changes in levels of cGMP, cAMP, CEA, CA-125 and PIINP could predict the clinical outcome after three months and 10 years. A fall in cGMP levels from the baseline sample to the three-month sample was found in patients with cancer of the uterine cervix with no signs of relapse after 10 years' observation. In agreement with previous reports (6, 7), it appears that cGMP levels in urine may predict relapse. The group of patients with ovarian cancer was too small for statistical analysis, but the results were consistent with previous studies (3-5) wherein the value of serial measurements of cGMP in urine have been thoroughly documented. The idea that cGMP and cAMP mediated opposing effects was forwarded (29), but no significant changes in cAMP have been found in urine from patients with malignant diseases (30). The present results support the view that cGMP, but not cAMP, may be a useful tumor marker.

CEA is an oncofetal antigen normally produced in the gastrointestinal tract and increased levels are observed in patients with malignancies in this organ. However, cancers of the ovaries and uterus are also associated with elevated CEA levels (12). In patients (n=122) with squamous cell carcinoma of the uterine cervix, the sensitivity and specificity of CEA determined prior to treatment were 46.3% and 63.1%, respectively (31). In the present study, the changes in CEA levels from the first to the second sample could not predict the clinical outcome after 10 years in any of the groups with gynecological cancers.

Since the introduction of CA-125, a high molecular weight glycoprotein, as a serum biomarker (32), it has been approved for monitoring recurrence of ovarian cancer (10). However, its usefulness has been questioned since significant elevations in CA-125 occur in 50 to 85% of the patients, and elevated CA-125 levels occur in a variety of both benign and malignant conditions (10). In the present study, the change in CA-125 levels did not show any statistical changes in the patients with cancers of the uterine cervix or corpus, and the small number of patients with ovarian cancers makes it impossible to conclude.

Amino terminal propeptide of type III procollagen (PIIINP) is a more recent marker which has been shown to have prognostic value in several gynecological malignancies (11). PIIINP a product of collagen metabolism is secreted into extracellular fluid when proliferation of fibroblast is increased. The changes in PIINP levels, from the baseline values to those three months after treatment, could not predict relapses.

The changes in values of CEA, CA-125 and PIINP from the baseline sample to the three-month sample did not predict long-term prognosis. However, serial measurements of these and other biomarkers have been established in clinical practice as a supplementary tool in the follow-up of these patients.

The present work supports the earlier findings that cGMP levels in urine may be useful in monitoring the effect of treatment in gynecological malignancies. The possibility exists that malignant cells try to escape the growth inhibitory effect of cGMP (33, 34) by an overexpression of cGMP transporters, as seen for leukemia (28). However, the increase in extracellular cGMP levels may simply represent overflow due to elevated activity of soluble guanylate cyclase secondary to increased nitric oxide synthase (NOS) activity in gynecologic cancers (35). The present observation that changes in extracellular cGMP levels shortly after treatment of gynecological cancers predict long-term prognosis indicates that nitric oxide pathways play an important role in the pathogenesis of these malignancies. With this background, the prognostic value of cGMP in extracellular fluids deserves further attention.

Acknowledgements

We thank the Norwegian Cancer Association for their financial support.

References

1 Guthrie D, Isah H, Latner AL and Turner GA: Urine cyclic GMP: A possible biochemical marker for cancer. IRCS Med Sci *7*: 209, 1979.

2 Turner GA, Ellis RD, Guthrie D, Latner AL, Ross WM and Skillen AW: Cyclic GMP in urine to monitor the response to ovarian cancer to therapy. Br J Obstet Gynaecol *89*: 760-764, 1982.

3 Luesley DM, Blackledge GR, Chan KK and Newton JR: Random urinary cyclic 3',5' guanosine monophosphate in epithelial ovarian cancer: relation to other prognostic variables and to survival. Br J Obstet Gynaecol *93*: 380-385, 1986.

4 Luesley DM, Chan KK, Newton JR and Blackledge GR: Serial urinary cyclic guanosine monophosphate measurements in the assessment of response to treatment in epithelial ovarian cancer. Br J Obstet Gynaecol *94*: 461-466, 1987.

5 Turner GA, Greggi S, Guthrie D, Benedetti PP, Ellis RD, Scambia G and Mancuso S: Monitoring ovarian cancer using urine cyclic GMP. A two-centre study. Eur J Gynaecol Oncol *11*: 421-427, 1990.

6 Turner GA, Ellis RD, Guthrie D, Latner AL, Monaghan JM, Ross WM, Skillen AW and Wilson RG: Urine cyclic nucleotide concentrations in cancer and other conditions; cyclic GMP: a potential marker for cancer treatment. J Clin Pathol *35*: 800-806, 1982.

7 Ørbo A, Jaeger R and Sager G: Urinary levels of cyclic guanosine monophosphate (cGMP) in patients with cancer of the uterine cervix: a valuable prognostic factor of clinical outcome? Eur J Cancer *34*: 1460-1462, 1998.

8 Ørbo A, Jaeger R and Sager G: Effect of serum and cell density on transmembrane distribution of cAMP and cGMP in transformed (C4-I) and non-transformed (WI-38) human cells. Int J Cancer *55*: 957-962, 1993.

9 Ørbo A, Jaeger R and Sager G: Cell density-dependence of cAMP and cGMP levels in four human cell lines derived from carcinomas of the uterine cervix. Gynecol Oncol *52*: 320-325, 1994.

10 Rapkiewicz AV, Espina V, Petricoin EF, III and Liotta LA: Biomarkers of ovarian tumours. Eur J Cancer *40*: 2604-2612, 2004.

11 Santala M, Simojoki M, Risteli J, Risteli L and Kauppila A: Type I and III collagen metabolites as predictors of clinical outcome in epithelial ovarian cancer. Clin Cancer Res *5*: 4091-4096, 1999.

12 Hammarstrom S: The carcinoembryonic antigen (CEA) family: structures, suggested functions and expression in normal and malignant tissues. Semin Cancer Biol *9*: 67-81, 1999.

13 Harper JF and Brooker G: Femtomole sensitive radioimmunoassay for cyclic AMP and cyclic GMP after 2'0 acetylation by acetic anhydride in aqueous solution. J Cyclic Nucl Res *1*: 207-218, 1975.

14 Skomedal T, Grynne B, Osnes JB, Sjetnan AE and Øye I: A radioimmunoassay for cyclic AMP (cAMP) obtained by acetylation of both unlabeled and labeled (3H-cAMP) ligand, or of unlabeled ligand only. Acta Pharmacol Toxicol *46*: 200-204, 1980.

15 Peracchi M, Lombardi L, Maiolo AT, Bamonti-Catena F, Toschi V, Chiorboli O, Mozzana R and Polli EE: Plasma and urine cyclic nucleotide levels in patients with acute and chronic leukemia. Blood *61*: 429-434, 1983.

16 Peracchi M, Lombardi L, Toschi V, Bamonti-Catena F, Mandelli V, Maiolo AT, Baldini L and Polli EE: Plasma and urine cyclic nucleotide levels in patients with neoplastic diseases. Cancer *54*: 3028-3034, 1984.

17 Peracchi M, Bamonti-Catena F, Lombardi L, Toschi V, Bareggi B, Cortelezzi A, Maiolo AT and Polli EE: Plasma cyclic nucleotide levels in monitoring acute leukemia patients. Cancer Detect Prev *8*: 291-295, 1985.

18 Peracchi M, Toschi V, Bamonti-Catena F, Lombardi L, Bareggi B, Cortelezzi A, Colombi M, Maiolo AT and Polli EE: Plasma cyclic nucleotide levels in acute leukemia patients. Blood *69*: 1613-1616, 1987.

19 Sager G: Cyclic GMP transporters. Neurochem Int *45*: 865-873, 2004.

20 Francis SH, Turko IV and Corbin JD: Cyclic nucleotide phosphodiesterases: relating structure and function. Prog Nucleic Acid Res Mol Biol *65*: 1-52, 2001.

21 Jedlitschky G, Burchell B and Keppler D: The multidrug resistance protein 5 functions as an ATP-dependent export pump for cyclic nucleotides. J Biol Chem *275*: 30069-30074, 2000.

22 Boadu E and Sager G: Reconstitution of ATP-dependent cGMP transport into proteoliposomes by membrane proteins from human erythrocytes. Scand J Clin Lab Invest *64*: 41-48, 2004.

23 Wielinga PR, van dH, I, Reid G, Beijnen JH, Wijnholds J and Borst P: Characterization of the MRP4-and MRP5-mediated transport of cyclic nucleotides from intact cells. J Biol Chem *278*: 17664-17671, 2003.

24 Chen ZS, Lee K and Kruh GD: Transport of cyclic nucleotides and estradiol 17-beta-d-glucuronide by multidrug resistance protein 4. Resistance to 6-mercaptopurine and 6-thioguanine. J Biol Chem *276*: 33747-33754, 2001.

25 Klokouzas A, Wu CP, van Veen HW, Barrand MA and Hladky SB: cGMP and glutathione-conjugate transport in human erythrocytes. Eur J Biochem *270*: 3696-3708, 2003.

26 van Aubel RA, Smeets PH, Van Den Heuvel JJ and Russel FG: Human organic anion transporter MRP4 (ABCC4) is an efflux pump for the purine end metabolite urate with multiple allosteric substrate binding sites. Am J Physiol Renal Physiol *288*: F327-F333, 2005.

27 Wu CP, Woodcock H, Hladky SB and Barrand MA: cGMP (guanosine 3',5'-cyclic monophosphate) transport across human erythrocyte membranes. Biochem Pharmacol *69*: 1257-1262, 2005.

28 Plasschaert SL, de Bont ES, Boezen M, vander Kolk DM, Daenen SM, Faber KN, Kamps WA, de Vries EG and Vellenga E: Expression of multidrug resistance-associated proteins predicts prognosis in childhood and adult acute lymphoblastic leukemia. Clin Cancer Res *11*: 8661-8668, 2005.

29 Goldberg ND, Haddox MK, Nicol SE, Glass DB, Sanford CH, Kuehl FA, Jr and Estensen R: Biologic regulation through opposing influences of cyclic GMP and cyclic AMP: the Yin Yang hypothesis. Adv Cyclic Nucleotide Res *5*: 307-330, 1975.

30 Hunt NH and Martin TJ: Hormone receptors and cyclic nucleotides; significance for growth and function of tumors. Mol Aspects Med *3*: 59-118, 1980.

31 Juang CM, Wang PH, Yen MS, Lai CR, Ng HT and Yuan CC: Application of tumor markers CEA, TPA, and SCC-Ag in patients with low-risk FIGO stage IB and IIA squamous cell carcinoma of the uterine cervix. Gynecol Oncol *76*: 103-106, 2000.

32 Bast Jr RC, Klug TL, St John E., Jenison E, Niloff JM, Lazarus H, Berkowitz RS, Leavitt T, Griffiths CT, Parker L, Zurawski VR Jr and Knapp RC: A radioimmunoassay using a monoclonal antibody to monitor the course of epithelial ovarian cancer. N Engl J Med *309*: 883-887, 1983.

33 Garg UC and Hassid A: Nitric oxide-generating vasodilators and 8-bromo-cyclic guanosine monophosphate inhibit mitogenesis and proliferation of cultured rat vascular smooth muscle cells. J Clin Invest *83*: 1774-1777, 1989.

34 Kariya K, Kawahara Y, Araki S, Fukuzaki H and Takai Y: Antiproliferative action of cyclic GMP-elevating vasodilators in cultured rabbit aortic smooth muscle cells. Atherosclerosis *80*: 143-147, 1989.

35 Thomsen LL, Lawton FG, Knowles RG, Beesley JE, Riveros-Moreno V and Moncada S: Nitric oxide synthase activity in human gynecological cancer. Cancer Res *54*: 1352-1354, 1994.

Received February 6, 2007
Revised March 29, 2007
Accepted April 4, 2007