

# Cyclic Adenosine 3',5'-Monophosphate-binding Proteins in Human Ovarian Cancer: Correlations with Clinicopathological Features

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## ABSTRACT

The regulatory subunits of protein kinase A, or cyclic AMP-binding proteins, were measured in a series of 107 human ovarian tumors (89 malignant, 7 borderline, and 11 benign tumors) and related to tumor clinicopathological features and patient survival.

Total cyclic AMP-binding protein levels were not significantly different between malignant tumors and either borderline or benign tumors. However, serous tumors showed significantly higher levels of total cyclic AMP-binding proteins than other malignant tumors ( $P = 0.007$ ). Poorly differentiated tumors also possessed significantly higher levels of binding proteins as compared with well/moderately differentiated tumors ( $P < 0.01$ ). Retrospective analysis of follow-up data also revealed a significant trend for patients with high tumor cyclic AMP-binding proteins to have poorer survival ( $P = 0.03$ ).

Individual binding proteins were identified by photoaffinity labeling, and the RI ( $M_r$  48,000) protein was expressed as a percentage of total cyclic AMP-binding proteins detected. The percentage of the RI protein was not significantly different among malignant, borderline, or benign pathologies and was not associated with tumor stage, differentiation, or debulk status. The percentage of RI was significantly increased in serous tumors compared to other common epithelial malignancies ( $P = 0.01$ ). In malignant tumors there was a significant positive correlation between the percentage of the RI protein and total cyclic AMP-binding proteins ( $P = 0.01$ ).

These data indicate that high tumor levels of cyclic AMP-binding proteins are associated with serous histology, poor differentiation, and poor patient survival.

## INTRODUCTION

Cyclic AMP exerts its major effects on cellular proliferation and differentiation via activation of cyclic AMP-dependent protein kinase A (1). The regulatory subunits of protein kinase A, often referred to as cyclic AMP-binding proteins, exist as different subtypes, types I and II, also known as RI and RII (2). High levels of tumor cyclic AMP-binding proteins may be associated with poor prognosis in patients with breast cancer (3, 4). The mechanisms by which high tumor cyclic AMP-binding protein levels confer poor prognosis is not clear, but it has been suggested that the RI ( $M_r$  48,000) and RII ( $M_r$  52,000; Ref. 5) regulatory proteins differentially regulate systems that control cellular proliferation and differentiation (6, 7).

Ovarian cancer is the most fatal of the gynecological malignancies, with a 5-year survival rate of around 20–30% (8), reflecting advanced disease at diagnosis. The limited success of conventional chemotherapeutic regimens has led to the investigation of novel targets for the treatment of this disease. Modulation of the expression of intracellular signal-transducing proteins provides the possibility of a biological approach to disease control. As we have recently reported, ovarian tumors exhibit differing levels of cyclic AMP-binding proteins and variations in the patterns of binding (5), and it was of interest to relate the levels and types of cyclic AMP-binding proteins to tumor clinicopathological features and patient survival.

## MATERIALS AND METHODS

**Patients.** Tissue samples from 107 patients were collected at initial debulking surgery for suspected ovarian malignancy. Upon collection the samples were stored in liquid nitrogen prior to subsequent analyses.

**Clinicopathological Details.** Tumor pathology, as obtained from patient records, was confirmed on hematoxylin and eosin stained sections. Tumors were classified as either malignant ( $n = 89$ ), borderline (low malignant potential;  $n = 7$ ), or benign ( $n = 11$ ) and assigned a histological type. Eighty-two of the malignant tumors were of common epithelial origin, and these could be subdivided into 38 serous, 22 endometrioid, 9 clear cell, 6 mucinous, and 7 undifferentiated tumors. Another seven malignant tumors were classified as one steroid cell tumor, one malignant mixed mesodermal tumor, two tumors of mixed epithelial origin, and three unclassified tumors. Of the seven borderline tumors, four were of serous origin and three of mucinous origin. Eleven benign tumors were classified into five mucinous cystadenofibromas, two serous cystadenofibromas, two fibroma/thecomas, one mature cystic teratoma, and one Brenner tumor.

Information was recorded on tumor stage (International Federation of Gynecology and Obstetrics) and differentiation. Early stage tumors were comprised of those presenting at stages I and II, and late stage were comprised of those presenting at stages III and IV. Well-differentiated and moderately differen-

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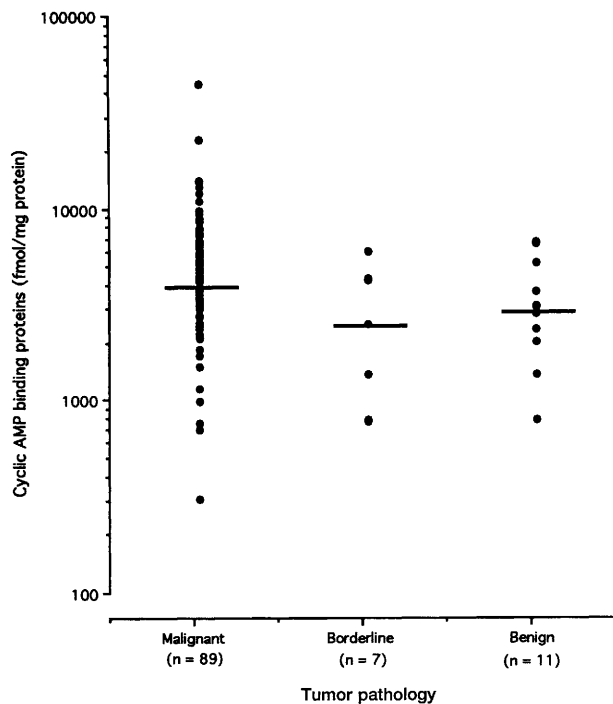


Fig. 1 Total cyclic AMP-binding protein levels in malignant, borderline, and benign ovarian tumors. No significant differences were observed between tumor groups. Horizontal bars, median values.

tiated tumors were considered as a single group because of the small number of tumors in each of these two categories and compared to those of poor differentiation. Postoperative tumor bulk was assessed by determining the greatest diameter of residual disease, where  $<2$  cm was classified as debulked and  $>2$  cm as nondebulked.

Patient survival times from operation were available for 87 patients (2 patients lost to follow-up) with malignant disease. Of these, postoperative therapy was given to 69 patients, consisting of 63 cisplatin and 6 chloroambucil chemotherapy regimens.

**Measurement of Total Cyclic AMP-binding Proteins.** Methodology for the measurement of cyclic AMP-binding proteins in ovarian tumors has been described previously (5). In brief, tumor cytosols were prepared by homogenization in a 20 mmol/liter Tris buffer (1:10 w/v) at  $0^{\circ}\text{C}$ , and the homogenate was centrifuged for 1 h at  $105,000 \times g$  at  $4^{\circ}\text{C}$ . Duplicate aliquots of the resulting supernatant (50  $\mu\text{l}$ ) were incubated at  $4^{\circ}\text{C}$  overnight with  $5',8',8'-^3\text{H}$  cyclic AMP (100  $\mu\text{l}$  25 nmol/liter; Amersham Life Science) in the absence or presence of varying amounts of radioinert cyclic AMP (Sigma) to give final concentrations of 0, 10, 20, 40, 80, and 10,000 nmol/liter. Bound cyclic AMP was separated from free nucleotide by filtration through Millipore filters (HAWP, 0.45  $\mu\text{m}$ ). The filters, absorbing protein-bound cyclic AMP, were washed and transferred to counting vials containing Micellar fluor NE260 scintillant (Nuclear Enterprises Technology Ltd.). The vials were incubated for 2 h at  $37^{\circ}\text{C}$ , and radioactivity was measured on a Tricarb liquid scintillation counter (Packard). The number of binding sites and dissociation constants of binding were determined by Scatchard

analysis (9), and binding was expressed as fmol of binding proteins per mg of cytosol protein (10).

**Photoaffinity Labeling.** Individual binding proteins were determined by photoaffinity labeling using 8-azidoadenosine  $3',5'$ -cyclic [ $^{32}\text{P}$ ]monophosphate (ICN Radiochemicals; Ref. 11). Cytosol samples (50  $\mu\text{l}$ ), prepared as described above, were incubated with  $8\text{-N}_3\text{-}^{32}\text{P}$ cyclic AMP (15  $\mu\text{l}$ , 0.4  $\mu\text{mol}$ /liter) and 0.27 M morpholino ethane sulfonic acid with 53 mM magnesium chloride (15  $\mu\text{l}$ ; Sigma) in 96-well microtiter plates at room temperature for 1 h in the dark. The reaction mixtures were then irradiated for 30 s at 254 nm by placing a Mineralight UVS-11 hand lamp directly over the plate. The reactions were stopped by the addition of SDS buffer (3% SDS, 15% 2-mercaptoethanol, 30 mM Tris, 30% glycerol, and 1% saturated bromophenol blue). The samples were heated to  $90^{\circ}\text{C}$  for 3 min, and the proteins were resolved electrophoretically on 12% SDS-PAGE with  $^{14}\text{C}$ -labeled molecular weight markers for 3 to 4 h at 35 mA. Following electrophoresis, the gels were fixed in 40% methanol, 10% acetic acid, 10% glycerol overnight, dried in a gel drier under vacuum, exposed to preflashed X-ray film (Kodak X-OMAT or Fuji) for 5 to 15 h at  $-80^{\circ}\text{C}$  in autoradiography cassettes fitted with intensifier screens and processed in Kodak X-ray developer and fixer. Autoradiograms were scanned by densitometry, and expression of the  $M_r$  48,000 protein was expressed as a percentage of the total scan.

**Statistics.** Relationships between variables were analyzed using the Mann-Whitney  $U$  and Kruskal-Wallis nonparametric tests. Correlations were analyzed using the Spearman rank test. Differences in survival were determined using the Kaplan-Meier method, and groups were compared using the log rank test and  $\chi^2$  test for trend.

## RESULTS

**Total Cyclic AMP-binding Proteins, Tumor Clinicopathological Features, and Patient Survival.** Cyclic AMP-binding proteins were detectable in all ovarian tumors studied irrespective of phenotype, levels varying between 284 and 41,458 fmol/mg protein. Although there was a subgroup of malignant tumors that contained very high levels of cyclic AMP-binding proteins, there was no significant difference in the median levels of binding proteins observed between malignant tumors and either borderline ( $P = 0.053$ ) or benign tumors ( $P = 0.07$ ; Fig. 1). Within the malignant tumor group, those of serous histology showed significantly higher levels of total cyclic AMP-binding proteins than endometrioid ( $P < 0.01$ ) or clear cell ( $P < 0.05$ ) tumors (Fig. 2). Serous tumors also expressed significantly higher levels of total cyclic AMP-binding proteins than other malignant tumor histologies combined ( $P = 0.007$ ; data not shown). A significant association was observed between total cyclic AMP-binding protein levels and tumor differentiation, with poorly differentiated tumors displaying higher binding protein levels ( $P < 0.01$ ; Fig. 3). Cyclic AMP-binding protein levels were not significantly different between early and late stage tumors ( $P = 0.27$ ) or between debulked and nondebulked tumors ( $P < 0.08$ ).

In order that tumor cyclic AMP-binding proteins might be related to patient survival, tumor cyclic AMP-binding protein

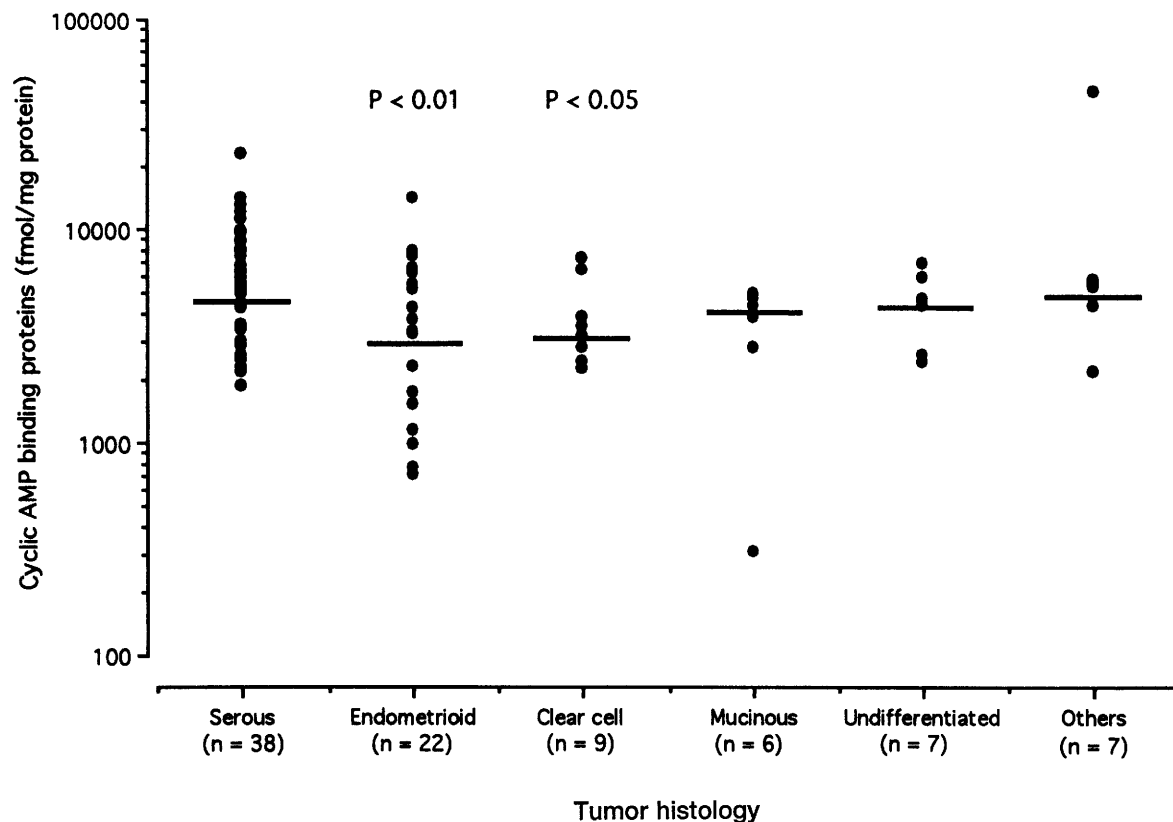


Fig. 2 Total cyclic AMP-binding protein levels in histological subgroups of malignant ovarian tumors. Serous tumors expressed significantly increased levels of cyclic AMP-binding proteins compared to tumors of endometrioid ( $P < 0.01$ ) or clear cell ( $P < 0.05$ ) histology. Horizontal bars, median values.

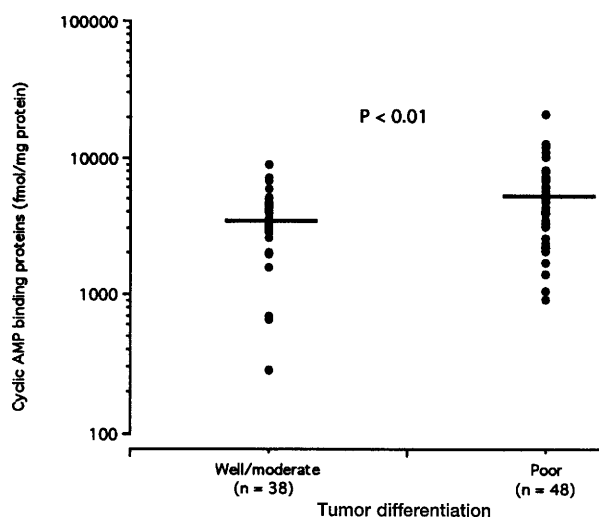


Fig. 3 Total cyclic AMP-binding protein levels in well/moderately and poorly differentiated ovarian tumors. Poorly differentiated tumors expressed significantly increased levels of cyclic AMP-binding proteins compared to tumors of well/moderate differentiation ( $P < 0.01$ ). Horizontal bars, median values.

levels were divided into three subgroups (<5000, 5000–7000, and >7000 fmol/mg protein). In patients with malignant ovarian tumors, there was a significant trend for high levels of tumor cyclic AMP-binding proteins to be associated with poorer survival (Fig. 4). Patients with tumor-binding protein levels in excess of 5000 fmol/mg cytosol protein showed significantly reduced survival ( $P < 0.03$ ).

Analysis of the three subgroups also showed that serous tumors were more likely to have cyclic AMP-binding protein levels in excess of 5000 fmol/mg protein than were other epithelial ovarian tumors ( $P = 0.01$ ; Table 1). In addition, poorly differentiated tumors ( $P = 0.001$ ) and those in which  $\geq 2$  cm of residual tumor remained following debulking surgery ( $P = 0.01$ ) were also more likely to have cyclic AMP-binding protein levels >5000 fmol/mg protein (Table 1).

**Type I Protein Kinase A, Total Cyclic AMP-binding Proteins, and Tumor Clinicopathological Features.** Photoaffinity labeling of cyclic AMP-binding proteins was performed in 79 malignant ovarian tumor cytosols. A significant positive correlation was observed between the percentage of binding detected as the RI protein, which migrated with a  $M_r$  48,000, and total cyclic AMP-binding proteins ( $P = 0.01$ ) in the malignant tumor group (Fig. 5).

The percentage expression of the RI protein was not significantly different among malignant, borderline, or benign tumor pathologies, and in malignant tumors was not associated

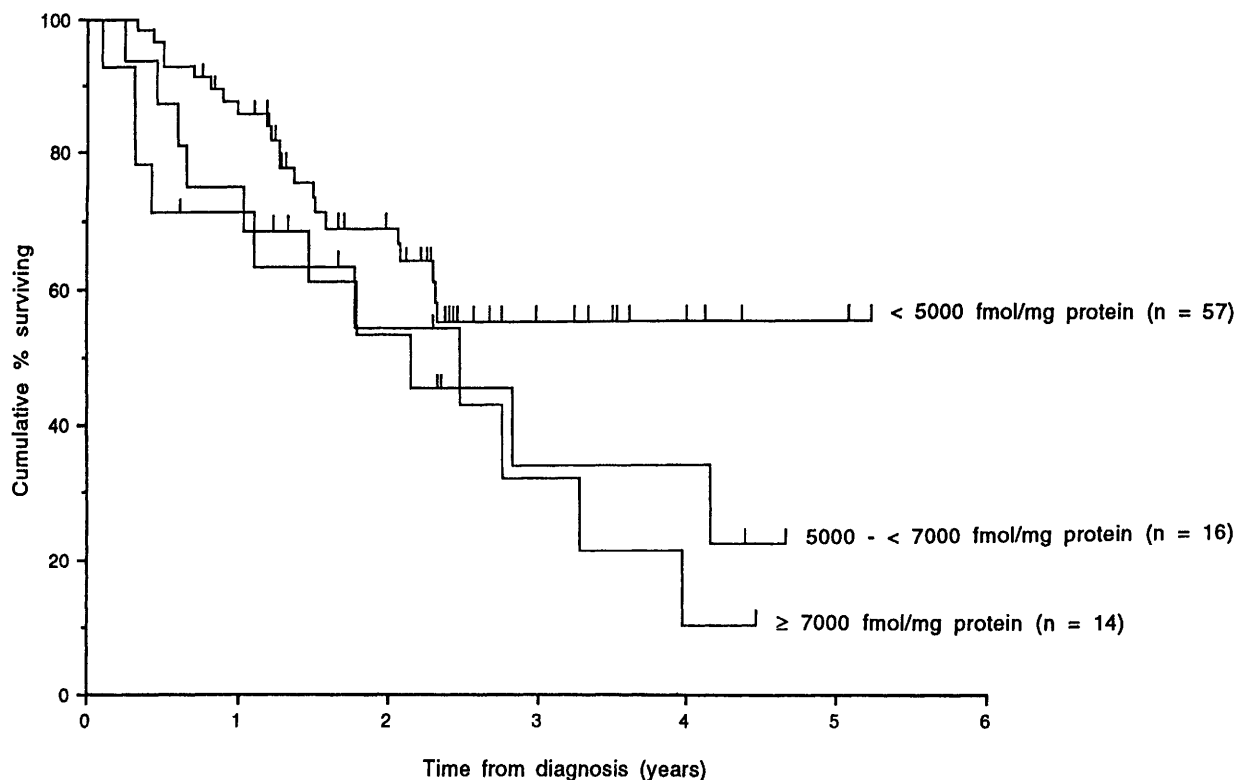


Fig. 4 Relationship between tumor cyclic AMP-binding protein levels and survival in patients with ovarian cancer. There was a significant trend for poor survival to be associated with high tumor cyclic AMP-binding protein levels ( $P = 0.03$ ).

Table 1 Total cyclic AMP-binding proteins and clinicopathological features

Variable	Classification	<5000 (fmol/mg protein)	5000-7000 (fmol/mg protein)	>7000 (fmol/mg protein)	$P^a$
Histology	Serous	20	7	11	0.01
	Rest	38	10	3	
Stage	I/II	22	3	4	0.29
	III/IV	36	13	10	
Grade	Well/moderate	33	3	2	0.001
	Poor	23	14	11	
Debulk status	<2 cm	43	7	6	0.01
	≥2 cm	14	9	7	

<sup>a</sup>  $P$  values determined by  $\chi^2$  test for trend.

with stage, differentiation, or debulk status (Table 2). Expression of the RI protein was not significantly increased in the serous group compared to other malignant histological subgroups combined ( $P = 0.057$ ; Table 2); however, this did achieve significance in the major subgroup of common epithelial tumors (excluding seven tumors of nonepithelial origin;  $P = 0.01$ ). No significant association was observed between expression of the RI protein and patient survival (data not shown).

## DISCUSSION

This is the first report in which cyclic AMP-binding proteins have been correlated with clinicopathological features of ovarian cancer. These data indicate that high tumor levels of

cyclic AMP-binding proteins are associated with a number of indicators of poor prognosis, including serous histology, poor tumor differentiation, and reduced patient survival, suggesting that therapeutic approaches to modulate cyclic AMP-binding protein levels may be beneficial in this disease.

Although there was no significant difference in the median levels of cyclic AMP-binding proteins among malignant, borderline, or benign tumors, there was a subgroup of malignant tumors in which cyclic AMP-binding protein levels were very high. With the exception of one very unusual steroid cell tumor, all tumors which expressed very high levels of cyclic AMP-binding proteins were of serous histology. Indeed, tumors of serous histology showed higher levels of cyclic AMP-binding proteins than did other histological subgroups. Poorly differentiated tumors, many of which were of serous histology, also displayed higher cyclic AMP-binding protein levels than did those of well/moderate differentiation, but no significant differences were observed between levels of binding proteins and tumor stage.

Interestingly, serous tumors also expressed a significantly higher percentage of the RI protein than other common epithelial malignancies, although this did not reach statistical significance compared to all other malignant ovarian tumors. A significant positive correlation was observed between the RI protein and total cyclic AMP-binding proteins in malignant ovarian tumor cytosols, where high binding was associated with a high proportion of the RI phenotype. This is in keeping with previous findings that malignant tumors overexpress RI at the

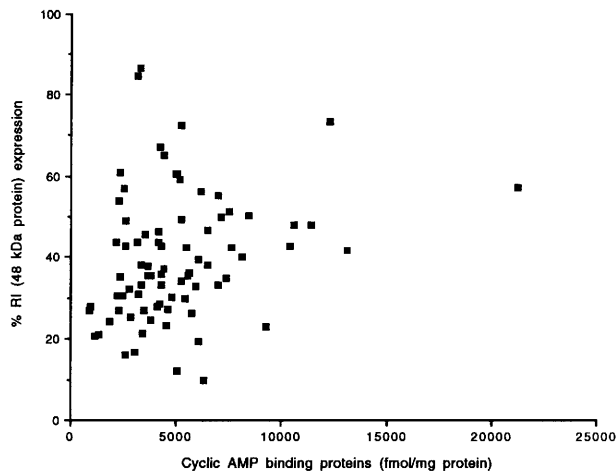


Fig. 5 A significant positive correlation was observed between total cyclic AMP-binding proteins and the percentage of the RI ( $M_r$  48,000) protein as detected by 8-azidoadenosine 3',5'-cyclic [ $^{32}$ P]monophosphate ( $P < 0.02$ ).

expense of RII (12). Expression of the RI protein was not significantly different in malignant tumors of varying stage and differentiation. Studies in gastric (13) and renal (14) cancers have also failed to demonstrate any association between RI and tumor differentiation. In colorectal cancers, however, poorly differentiated tumors have been shown to be associated with the overexpression of RI relative to well/moderately differentiated tumors, but no association between RI and stage was observed (15).

Survival analyses indicated that in a cohort of 87 ovarian cancer patients, there was a significant trend for higher tumor cyclic AMP-binding protein levels to be associated with poorer survival. From retrospective analyses, we observed that 35% of the patients had tumors with binding protein levels in excess of 5000 fmol/mg cytosol protein, and that these patients had a reduced survival compared to the rest. Furthermore, high cyclic AMP-binding protein levels, in excess of 5000 fmol/mg protein, were also associated with a number of indicators of poor prognosis, including serous histology, poor tumor differentiation, and nonbulked status. To determine whether cyclic AMP-binding protein levels are truly independent prognostic factors in ovarian cancer, multivariate analysis would have to be performed. We feel, however, that because our results come from a retrospective trawl of the data, and the fact that patient numbers involved in the current survival study are small, it would be premature to comment on the potential prognostic significance of cyclic AMP-binding protein levels in ovarian cancer. It is of particular interest, however, that an association was observed between survival status and high cyclic AMP-binding protein levels in patients with stage I disease. The prognosis for patients with stage I ovarian cancer, in which the disease is localized to the ovaries and surgery is usually effective, is good. In this study, three stage I patients who did not survive showed tumor cyclic AMP-binding protein levels in excess of 7000 fmol/mg protein, suggesting that, even in stage I disease, high cyclic AMP-binding protein levels may be associated with aggressive tumor behavior.

Table 2 Percentage of expression of RI ( $M_r$  48,000 protein) and tumor clinicopathological features

Clinicopathology	Median (range)	$P^a$
Pathology		
Malignant	35.9 (8.5–85.2)	
Borderline	37.0 (28.8–51.7)	0.08
Benign	42.3 (5.9–70.4)	
Histology		
Serous	43.4 (11.0–72.0)	
Rest <sup>b</sup>	34.1 (8.5–85.2)	0.06 <sup>c</sup>
Stage		
I/II	34.5 (11.0–85.2)	
III/IV	37.6 (8.5–83.3)	0.93
Differentiation		
Well/moderate	33.4 (11–85.2)	
Poor	38.2 (8.5–72.0)	0.59
Debulk status		
<2 cm	36.7 (11.0–85.2)	
≥2 cm	36.0 (14.9–63.8)	0.29

<sup>a</sup>  $P$  values were determined by Kruskal-Wallis and Mann-Whitney  $u$  tests.

<sup>b</sup> Rest, all malignant tumor histologies excluding serous.

<sup>c</sup> Serous *versus* all other common epithelial tumors,  $P = 0.01$ .

Observations in breast cancer have also implicated a link between tumor cyclic AMP-binding protein levels and eventual outcome of the disease (3, 4). In these studies, patients who had breast tumor cyclic AMP-binding proteins in excess of 8000 fmol/mg cytosol protein (9–12% of the patients) had an increased chance of disease recurrence compared to patients with lower cyclic AMP-binding protein levels. In light of the findings in breast cancer, our observations in ovarian cancer merit a prospective study to confirm the implication that high tumor cyclic AMP-binding protein levels confer poor prognosis in this disease.

Interestingly, patient survival was not associated with the percentage of total cyclic AMP-binding which was detected as the RI ( $M_r$  48,000) protein by photoaffinity labeling. Although we observed a significant positive correlation between the percentage of binding detected as the RI protein and total cyclic AMP-binding proteins, this was by no means absolute, and in a Kaplan-Meier analysis total cyclic AMP-binding proteins was more influential than the percentage of RI. This is not necessarily incompatible with the hypothesis that overexpression of RI is associated with aggressive tumor behavior. Indeed, this may be because total binding, rather than the percentage of RI within the total binding, is a better reflection of overexpression of RI.

The prognosis for patients with cancer of the ovary depends on a number of interrelated factors including tumor stage, histological type, and differentiation, and the amount of residual tumor bulk following surgery (16). However, how the biological behavior of the tumor influences these prognostic factors, *e.g.*, the possibility of successful debulking, is not known. We have presented data which, by univariate analysis, suggest a relationship between high tumor cyclic AMP-binding proteins and poor patient survival. We have also demonstrated that high tumor cyclic AMP-binding proteins are associated with other tumor clinicopathological features, including histology and differentiation. Indeed, poorly differentiated serous tumors are among the most common types of malignant epithelial ovarian tumors and are characterized by their particularly aggressive behavior. In

light of this, modulation of the expression of cyclic AMP-binding proteins may provide a possible biological approach to the control of this disease. Strategies to interfere with cyclic AMP-binding proteins, *e.g.*, 8-chloro cyclic AMP, a cyclic AMP analogue which modulates the RI subunit (7), have been shown to cause growth inhibition in a number of cancer cell lines (17, 18) and also in nude mice xenograft models (12, 19, 20). Phase I clinical trials of this drug are currently under way (21, 22). Down-regulation of RI using antisense oligodeoxynucleotides has also produced growth inhibition both *in vitro* (23) and *in vivo* (24). We are currently exploring these methodologies as potential therapeutic strategies in ovarian cancer.

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## REFERENCES

- Walsh, D. A., Perkins, J. P., and Krebs, E. G. An adenosine 3':5'-monophosphate dependent protein kinase from rabbit skeletal muscle. *J. Biol. Chem.*, *243*: 3763-3765, 1968.
- Reimann, E. M., Walsh, D. A., and Krebs, E. G. Purification and properties of rabbit skeletal muscle adenosine 3',5'-cyclic monophosphate-dependent protein kinases. *J. Biol. Chem.*, *246*: 1986-1995, 1971.
- Miller, W. R., Elton, R. A., Dixon, J. M., Chetty, U., and Watson, D. M. A. Cyclic AMP binding proteins and prognosis in breast cancer. *Br. J. Cancer*, *61*: 263-266, 1990.
- Miller, W. R., Watson, D. M. A., Jack, W., Chetty, U., and Elton, R. A. Tumour cyclic AMP binding proteins: an independent prognostic factor for disease recurrence and survival in breast cancer. *Breast Cancer Res. Treat.*, *26*: 89-94, 1993.
- Ramage, A. D., Burns, D. J., and Miller, W. R. Cyclic adenosine 3',5'- monophosphate-binding proteins in human ovarian cancers. *Br. J. Cancer*, *69*: 186-190, 1994.
- Cho-Chung, Y. S. Differential therapy of cancer targeting the RI $\alpha$  regulatory subunit of cAMP-dependent protein kinase (Review). *Int. J. Oncol.*, *3*: 141-148, 1993.
- Cho-Chung, Y. S., Clair, T., Tortora, G., and Yokozaki, H. Role of site-selective cAMP analogs in the control and reversal of malignancy. *Pharmacol. & Ther.*, *50*: 1-33, 1991.
- Scully, S. R. Pathology of ovarian carcinoma. *In*: S. M. Piver, (ed.), *Ovarian Malignancies*, pp. 72-95. Edinburgh: Churchill Livingstone, 1987.
- Scatchard, F. The attraction of proteins for small molecules and ions. *Ann. N.Y. Acad. Sci.*, *51*: 660-672, 1949.
- Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.*, *72*: 248-254, 1976.
- Pomerantz, A. H., Rudolph, S. A., Haley, B. E., and Greengard, P. Photoaffinity labelling of a protein kinase from bovine brain with 8-azidoadenosine 3',5' monophosphate. *Biochemistry*, *14*: 3858-3862, 1975.
- Ally, S., Tortora, G., Clair, T., Grieco, D., Merlo, G., Katsaros, D., Ogreid, D., Døskeland, S. O., Jahnsen, T., and Cho-Chung, Y. S. Selective modulation of protein kinase isozymes by the site-selective analog 8-chloroadenosine 3',5'-cyclic monophosphate provides a biological means for control of human colon cancer cell growth. *Proc. Natl. Acad. Sci. USA*, *85*: 6319-6322, 1988.
- Yasui, W., Sumiyoshi, H., Ochiai, A., Yamahara, M., and Tahara, E. Type I and II cyclic adenosine 3',5'- monophosphate-dependent protein kinase in human gastric mucosa and carcinomas. *Cancer Res.*, *45*: 1565-1568, 1985.
- Fossberg, T. M., Døskeland, S. O., and Ueland, P. M. Protein kinases in human renal cell carcinoma and renal cortex. *Arch. Biochem. Biophys.*, *189*: 272-281, 1978.
- Bradbury, A. W., Carter, D. C., Miller, W. R., Cho-Chung, Y. S., and Clair, T. Protein kinase-a (PK-A) regulatory subunit expression in colorectal-cancer and related mucosa. *Br. J. Cancer*, *69*: 738-742, 1994.
- Ansell, S. M., Rapoport, B. L., Falkson, G., Raats, J. I., and Moeken, C. M. Survival determinants in patients with advanced ovarian cancer. *Gynecol. Oncol.*, *50*: 215-220, 1993.
- Tagliaferri, P., Katsaros, D., Clair, T., Ally, S., Tortora, G., Neckers, L., Rubalcava, B., Parandoosh, Z., Chang, Y. A., Revankar, G. R., Crabtree, G. W., Robins, R. K., and Cho-Chung, Y. S. Synergistic inhibition of growth of breast and colon human cancer cell lines by site-selective cyclic AMP analogs. *Cancer Res.*, *48*: 1642-1650, 1988.
- Tortora, G., Tagliaferri, P., Clair, T., Colamonic, O., Neckers, L. M., Robins, R. K., and Cho-Chung, Y. S. Site-selective cyclic AMP analogs at micromolar concentrations induce growth arrest and differentiation of acute promyelocytic, chronic myelocytic and acute lymphocytic human leukemia cell lines. *Blood*, *71*: 230-233, 1988.
- Parandoosh, Z., Rubalcava, B., Finch, R. A., Robins, R. K., and Avery, T. L. Changes in diacylglycerol and membrane associated protein kinase C activity reflect the growth status of xenografted human mammary carcinoma treated with 8-Cl-cAMP. *Cancer Lett.*, *49*: 195-200, 1990.
- Ramage, A. D., Langdon, S. P., Ritchie, A. A., Burns, D. J., and Miller, W. R. Growth inhibition by 8-chloro cyclic AMP of human HT29 colorectal and ZR-75-1 breast carcinoma xenografts is associated with selective modulation of protein kinase A isoenzymes. *Eur. J. Cancer*, *31*: 969-973, 1995.
- Saunders, M. P., Salisbury, A. J., Harris, A. L., Long, L., O'Byrne, K. J., Macaulay, V. M., Miki, K., Cho-Chung, Y. S., and Talbot, D. C. Phase I study of the protein kinase A regulator 8-chloro cyclic AMP. *Proc. Am. Assoc. Cancer Res.*, *36*: 241, 1995.
- Tortora, G., Ciardiello, F., Pepe, S., Tagliaferri, P., Ruggiero, A., Bianco, C., Guarrasi, K., and Bianco, A. R. Phase I clinical study with 8-chloro-cAMP and evaluation of immunological effects in cancer patients. *Clin. Cancer Res.*, *1*: 377-384, 1995.
- Yokozaki, H., Budillon, A., Tortora, G., Meissner, S., Beucage, S. L., Miki, K., and Cho-Chung, Y. S. An antisense oligodeoxynucleotide that depletes RI $\alpha$  subunit of cyclic AMP-dependent protein kinase induces growth inhibition in human cancer cells. *Cancer Res.*, *53*: 868-872, 1993.
- Clair, T., Yokozaki, H., Tortora, G., Meissner, S., Beucage, S. L., and Cho-Chung, Y. S. An antisense oligodeoxynucleotide targeted against the type I regulatory subunit (RI $\alpha$ ) mRNA of cAMP-dependent protein kinase A (PKA) inhibits the growth of LS-174T human colon carcinoma in athymic mice. *Proc. Am. Assoc. Cancer Res.*, *32*: 277, 1991.