Growth Inhibition of Human Ovarian Cancer Cells by Differential Modulation of Protein Kinase A Isozymes

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We examined the effect of modulation of PKA isozymes on the growth of human ovarian cancer cells. Three ovarian cancer cell lines, 2774, SK-OV-3, and OVCAR-3, were examined in this study. The treatment of 5 μM 8-Cl-cAMP, which has been known to down-regulate RI (or type I PKA) and up-regulate RII (or type II PKA), markedly inhibited the growth of all cell lines (50-80% at day 6). To test whether alteration in PKA regulatory subunits level can change the growth characteristics of ovarian cancer cells, we introduced RIIβ- expression construct and RIα antisense-expression construct into 2774 cells. The overexpression of RIIβ down-regulated RIα protein, and the antisense-expression of RIα up-regulated RIIβ protein, showing that the intracellular levels of RI and RII are reciprocally regulated. In both cases, cell growth was reduced by 30% at day 2. These results indicate that the growth of ovarian cancer cells is controlled by the signals from PKA isozymes, and the modulation of PKA isozymes can be employed for the human ovarian cancer therapy.

Cyclic AMP (cAMP), initially discovered as an intracellular signal transducer or second messenger (Sutherland and Rall, 1957), has been considered to mediate growth-controlling signals. In mammalian cells, cAMP acts mainly by binding to protein kinase A (PKA). There are two types of PKA, type I and type II, which share common catalytic (C) subunits, but are distinguished by the association of their regulatory (R) subunits, RI and RII, respectively. Four genetically distinct R subunit isoforms (RIα, RIβ, RIIα, and RIIβ) and three C subunit isoforms (Cα, Cβ, and Cγ) have been identified (McKnight et al, 1988).

Relationship between the differential expression of two types of PKA and cell growth/differentiation has been studied in a variety of systems (Cho-Chung, 1990). When the cells undergo active proliferation or are in an early developmental stage, high type I PKA activity and RI/RII ratio are observed. However, terminally differentiated and non-growing cells show a high type II PKA activity and a low RI/RII ratio.

Previous reports suggested that the actions of PKA isozymes might also be involved in the differentiation of ovarian cells. It was demonstrated that PKA activity in rat ovary increases progressively during postnatal development (DeAngelo et al., 1975). PKA isozyme distribution also changes during the ovarian development. Ovarian cytosol of 6-day-old neonates contains largely type I PKA, whereas in 34-day-old or older rats, type II PKA predominates (Hunzicker-Dunn, 1982). Thus, two PKA isozymes may play different roles in the early development of rat ovaries.

Since PKA-mediated events seem to be involved in the differentiation process of ovarian cells, we hypothesized that the differential modulation of PKA isozymes would affect the growth of ovarian cancer cells. In the present study, we employed three human ovarian cancer cell lines to test whether the cAMP analogue, which has been known to inhibit the growth of other cancer cells by differentially modulating PKA isozymes, could inhibit the growth of human ovarian cancer cells. We also examined the effect of up- or down-regulation of PKA regulatory subunits on the ovarian cancer cell growth.

Materials and Methods

Cell culture

SK-OV-3 cells were maintained in Dulbecco's modified Eagle's medium. 2774 and OVCAR-3 cells were grown in RPMI 1640. All the media were supplemented with 10% heat-inactivated fetal bovine serum (GIBCO-BRL), 100 units/ml penicillin G, 100 μg/ml streptomycin. For OVCAR-3 cells medium was supplemented with 10 μg/ml insulin. Cells were maintained in a humidified atmosphere of 5% CO_2 at 37℃.

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Construction of expression vectors and introduction into 2774 cells

OT1521 retroviral vector (McGeady et al., 1989) was used to generate expression constructs. The constructs for the overexpression of RIIβ (Tortora and Cho-Chung, 1990a) and antisense expression of RIα (Tortora et al., 1994) were described previously. Retroviral constructs were introduced into 2774 human ovarian cancer cells using replication-deficient retrovirus system and the selections of high expressor clonal lines were performed as described previously (Kim et al., 1996).

Western blot analysis

Cells were treated with 0 to 120 μM $ZnSO_4$ for two d to induce the expression of various constructs before analysis. Cell extracts were prepared as described (Tortora et al., 1990b). Western blot analysis was performed as before (Kim et al., 1996) using ECL detection system (Amersham).

Monolayer growth assay

To examine the growth of ovarian cancer cells, we used microculture tetrazolium assay (Alley et al., 1988). Five thousand cells in 0.1 ml of culture medium were seeded on each well of flat-bottomed 96-well plate. After 8 h, 0.1 ml of culture medium containing additives was added to each well. To examine the effect of 8-Cl-cAMP, final 5 μM 8-Cl-AMP was used, and for the induction of introduced genes, cells were treated with $ZnSO_4$ at a final concentration of 60 μM. To determine the cell growth, 50 μl of 2 mg/ml MTT (3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) was added to each well. After 4 h incubation, the medium was removed and the colored material was dissolved in 150 μl of dimethyl sulfoxide. The absorbance at a wavelength of 570 nm was measured using a microplate reader (BIO-RAD).

Results

Effects of 8-Cl-cAMP on the growth of ovarian cancer cells

In an attempt to look at the effect of differential modulation of PKA isozymes on human ovarian cancer cell growth, we examined the changes in the growth rate of three lines of ovarian cancer cells, 2774, SK-OV-3, and OVCAR-3, after the treatment of a cAMP analogue. In the present study, we used 8-Cl-cAMP to modulate PKA isozymes, because it is known to inhibit growth of cancer cells by down-regulation of type I PKA activity and up-regulation of type II PKA activity (Rohlff et al., 1993). Thus, effect of 8-Cl-cAMP on the cell growth may well represent the relationship between the signals from PKA isozymes and the cell growth.

Treatment of 5 μM 8-Cl-cAMP on ovarian cancer cells brought about 50-80% growth inhibition at day 6

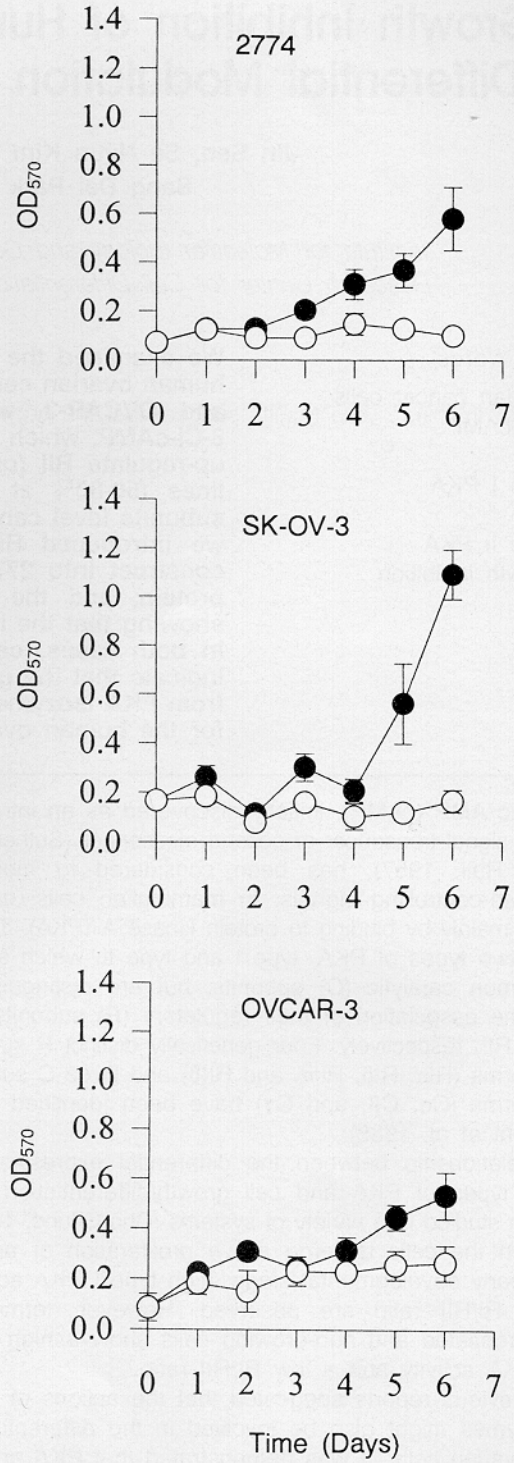

Fig. 1. Effects of 8-Cl-cAMP treatment on the cell growth of three ovarian cancer cell lines. Cells were cultured in the 96-well plate, and their growth rates were examined with a microculture tetrazolium assay. Absorbance at the wavelength of 570 nm was determined at the time of treatment (day 0) and every 24 h thereafter up to day 6. Closed circle (●) indicates untreated cells and open circle (○) indicates cells treated with 5 μM 8-Cl-cAMP. Data indicate mean ± S.D. of 6 separate determinations.

(Fig. 1). The level of growth inhibition was similar in 2774 and SK-OV-3 (about 80%) and was smaller in

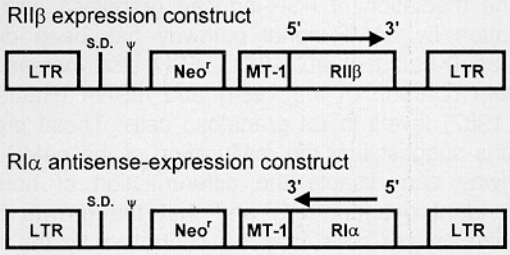

Fig. 2. Schematic diagram of the expression constructs for RIIβ and RIα antisense. LTR, long terminal repeat from moloney murine sarcoma virus (MSV). S.D., splicing donor site. ψ, packaging site. Neo^r, neomycin phosphotransferase gene which confers G418 resistance. MT-1, mouse metallothionein-1 promoter. Arrows indicate the orientation of sense-strand.

OVCAR-3 (50%). These results suggest that the growth of ovarian cancer cells could be regulated by differential modulation of PKA isozymes with a cAMP analogue.

Introduction of RIIβ- and RIα antisense-expression constructs into ovarian cancer cells

Since the growth of ovarian cancer cells was inhibited by 8-Cl-cAMP treatment, we hypothesized that the down-regulation of type I PKA and/or the up-regulation of type II PKA would induce growth inhibition of ovarian cancer cells. To test this hypothesis, we introduced the expression construct for RIIβ and the antisense-expression construct for RIα into 2774 human ovarian cancer cells. Human RIIβ (Levy et al., 1988) and RIα (Sandberg et al., 1987) cDNAs were inserted into the OT1521 (McGeady et al., 1989) retroviral vector in sense and antisense orientations, as shown in Fig. 2. OT1521 vector contains the neomycin phosphotransferase gene, which allows the selection of stable infectants by G418 selection, and the mouse metallothionein-1 promoter, which enables the inducible expression of the inserted gene upon treatment with $CdCl_2$ or $ZnSO_4$. The expression constructs were introduced into the 2774 ovarian cancer cells and individual clones were isolated as described previously (Kim et al., 1996). The expressions of introduced genes were checked by Northern or Western analyses. Single high expressor clone that showed the highest level of RIIβ mRNA in Northern analysis was selected as a 2774-RIIβ. For RIα antisense-expression, RIα antisense-infected cells were screened for low expression of RIα in Western analysis. A clone of the lowest expression of RIα protein was selected as a 2774-RIα-r (Data not shown).

Overexpression of RIIβ and the effect on the RIα level

To find out the optimum induction condition for the expression of introduced genes, we examined the toxicity of $ZnSO_4$ at various concentrations. Up to 120 μM, $ZnSO_4$ did not exert any effect on the growth of 2774 cells (data not shown). Thus, 0 to 120 μM $ZnSO_4$ was used to induce the introduced genes in further experiments.

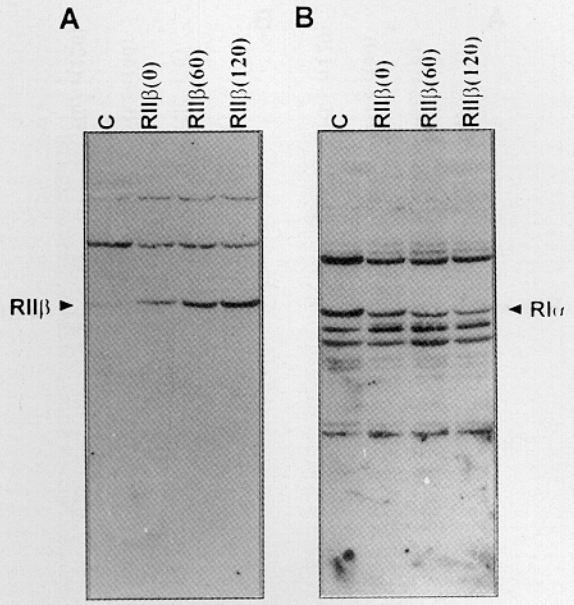

Fig. 3. Protein levels of RIIβ and RIα in 2774-RIIβ. Total cell extracts from 2774-RIIβ were analyzed with Western blot analysis as described in "Materials and Methods". RIIβ (panel A) and RIα (panel B) proteins were detected with polyclonal antiserum generated against recombinant RIIβ and RIα proteins produced in bacteria, respectively. RIIβ and RIα bands were indicated with arrowheads. C, parental cells; RIIβ (0) 2774-RIIβ cells not treated with $ZnSO_4$, RIIβ (60), and 2774-RIIβ cells treated with 60 μM $ZnSO_4$; RIIβ (120) and 2774-RIIβ cells treated with 120 μM $ZnSO_4$.

The expressions of RIIβ in the 2774-RIIβ at several concentrations of $ZnSO_4$ were compared with that of parental 2774 cells (Fig. 3A). The 2774 cells exhibited very low, but detectable level of RIIβ, as reported previously (Seo et al., 1995). In 2774-RIIβ cells, 7-, 12-, and 20-fold increases in RIIβ level were observed at the induction conditions of 0, 60, and 120 μM $ZnSO_4$, respectively, as determined by Western analysis and densitometric scanning. The increase of RIIβ protein brought about the decrease of RIα (Fig. 3B). When RIIβ expression was induced with 120 μM $ZnSO_4$, RIα protein was decreased up to 30% of the parental cells. Even without the induction, RIα decreased to 50% of the control cells showing that increase in RIIβ down-regulated RIα.

Reduction of RIα in 2774-RIα-r cells and the effect on the RIIβ level

The expressions of RIα and RIIβ were examined in 2774-RIα-r cells by Western blot analysis. At all induction conditions (0, 60, and 120 μM $ZnSO_4$), 2774-RIα-r showed 30% decreased RIα level compared to that of parental cells (Fig. 4B). RIIβ protein was increased slightly (10-30%) at all induction conditions (Fig. 4A).

Monolayer growth

To examine the effect of the modulation of RI and RII levels on the ovarian cancer cell growth, we compared the growth rates of 2774-RIIβ and 2774-RIα-r with that

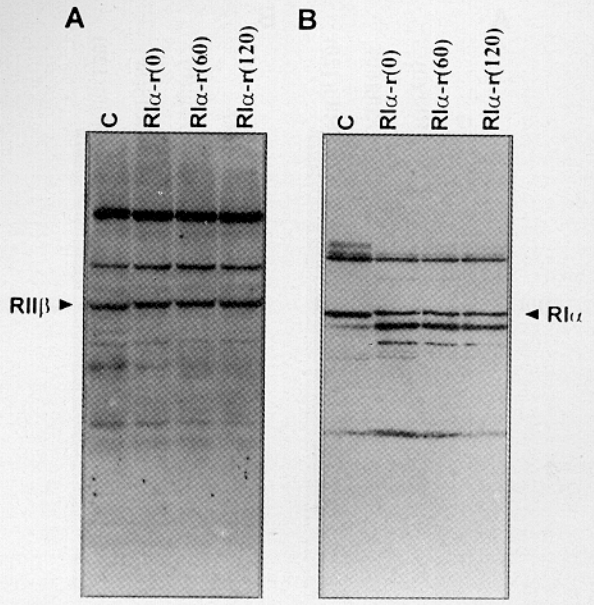

Fig. 4. Protein levels of RIIβ and RIα in 2774-RIα-r. Total cell extracts of 2774-RIα-r were analyzed with Western blot analysis as described in "Materials and Methods". RIIβ (panel A) and RIα proteins (panel B) were detected with the same antibody as described in Fig. 3. RIIβ and RIα bands were indicated with arrowheads. C, parental cells. RIα-r (0) 2774-RIα-r cells not treated with $ZnSO_4$; RIα-r (60) and 2774-RIα-r cells treated with 60 μM $ZnSO_4$; RIα-r (120) and 2774-RIα-r cells treated with 120 μM $ZnSO_4$.

of parental cells. The growth of 2774-RIIβ and 2774-RIα-r was reduced by 30% at day 2 as compared with parental cells. These data show that the up-regulation of RIIβ and/or down-regulation of RIα can induce growth inhibition in human ovarian cancer cells.

Discussion

In the present study, we have shown that 1) 8-Cl-cAMP induces growth inhibition in three lines of ovarian cancer cells, 2) overexpression of RIIβ down-regulates RIα in a quantity-dependent manner, 3) down-regulation of RIα increases the level of RIIβ, and 4) up-regulation of RIIβ and/or down-regulation of RIα causes growth inhibition of ovarian cancer cells.

The close relationship between the activities of PKA isozymes and the cell growth has been well established in other systems (Cho-Chung, 1990). It has been reported that down-regulation of type I PKA (or RIα) and up-regulation of type II PKA (or RIIβ) by cAMP analogues such as 8-Cl-cAMP brings about growth inhibition and differentiation in a variety of cancer cell lines (Katsaros et al., 1987; Tortora et al., 1989a). Suppression of type I PKA or RIα by the treatment of RIα antisense oligodeoxynucleotide also induced an increase of RIIβ and type II PKA activity, and inhibited the growth of cancer cells (Tortora et al., 1991; Yokozaki et al., 1993). However, the effects of the modulation of PKA isozymes on the ovarian cancer cell growth have not been well examined.

The mediation of FSH-induced granulosa cell differentiation by cAMP-signal pathway has been demonstrated (Knecht et al., 1981). FSH also increases RII protein (Darbon et al., 1984) and mRNA (Ratoosh et al., 1987) levels in rat granulosa cells. These previous reports suggest that the malfunction of the cAMP signal pathway can disable the differentiation of hormone-dependent ovarian cells and that the growth of the ovarian cancer cells may be controlled by the modulation of PKA signal pathway.

The growth of ovarian cancer cells was markedly inhibited (50-80%) by 8-Cl-cAMP treatment (Fig. 1). The effects of 8-Cl-cAMP treatment have been examined in a variety of cancer cells or transformed cells: The treatment of 8-Cl-cAMP 1) induces the nuclear translocation of RII into the nucleus (Clair et al., 1987; Ally et al., 1988; Ally et al., 1989), 2) down-regulates RI and up-regulates RII in mRNA and protein levels (Ally et al., 1988; Ally et al., 1989; Tortora et al., 1989a, b; Rohlff et al., 1993), 3) down-regulates type I PKA activity and up-regulates type II PKA activity (Rohlff et al., 1993), 4) increases cAMP-responsive element binding activity of nuclear extracts (Mednieks et al., 1989), and 5) reduces the expression of oncogenes such as c-*myc* (Ally et al., 1989; Tortora et al., 1989a), and N-*ras* (Ally et al., 1989) and oncogene products, transforming growth factor α and $p21^{ras}$ (Tortora et al., 1989b). Also, the differentiation of HL60 cells by 8-Cl-cAMP was abrogated by the treatment of RIIβ antisense oligodeoxynucleotide (Tortora et al., 1990b), showing that the RIIβ protein or type II PKA takes an important part in the differentiation induced by 8-Cl-cAMP. These previous reports suggest that the growth inhibition brought about by 8-Cl-cAMP treatment in the ovarian cancer cells could be mimicked by the up-regulation of type II PKA and down-regulation of type I PKA.

Growth inhibition of cancer cells or transformed cells by the overexpression of RIIβ has been reported previously (Budillon et al., 1995; Kim et al., 1996; Nesterova et al., 1996). However, the overexpression of RIIβ has not been tried in the hormone-dependent ovarian cells. The results obtained in the present study showed that overexpression of RIIβ also induces growth inhibition in ovarian cancer cells (Fig. 5). Effects of RIα antisense-expression were also of our interest because down-regulation of type I PKA by RIα antisense-expression in SK-N-SH human neuroblastoma cells could not induce growth inhibition (Kim et al., 1997). In 2774 ovarian cancer cells, RIα-antisense expression as well as RIIβ overexpression resulted in marked growth inhibition (Fig. 5). Although we are not aware of the causative physiological difference between the neuroblastoma cells and the ovarian cancer cells, we suspect that the endogenous PKA isozyme status may be responsible in part for the disparity. In the case of neuroblastoma cells, there was no detectable endogenous RIIβ mRNA or protein, and RIIβ was not induced by the down-regulation of RIα. However, the

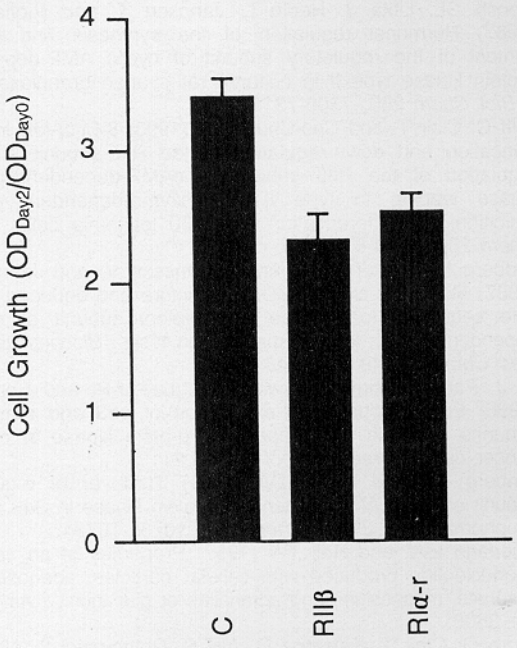

Fig. 5. Effects of RIIβ overexpression and RIα antisense expression on the growth of 2774 cells. The monolayer growth rates of parental cells (C), 2774-RIIβ (RIIβ), and 2774-RIα-r (RIα-r) were examined as described in "Materials and Methods". Cell growth was determined with a microculture tetrazolium assay and shown as OD_{570} at day 2 divided by OD_{570} at day 0. Data express mean±S.D. of 6 separate determinations.

basal level of RIIβ could be observed in ovarian cancer cells, and RIIβ was increased by the down-regulation of RIα. Thus, the basal level and/or increase of RIIβ might be necessary for the growth inhibition by the down-regulation of RIα.

Overexpression of RIIβ in 2774 cells resulted in the decrease of RIα (Fig. 3). The reciprocal regulation between RI and RII has been studied by the overexpression of PKA subunits. Overexpression of RIIα in the ras-transformed NIH3T3 cells eliminated type I PKA, whereas the overexpression of RI did not alter PKA isozymes distribution (Otten and McKnight, 1989), suggesting that RII has higher affinity toward C subunit than that of RI. Overexpression of RII with high affinity leaves RI as free subunits, which are subject to degradation (Steinberg and Agard, 1981).

The increase of RII by the decrease of RIα has also been reported. In the studies using antisense oligodeoxynucleotides, the decrease of RIα was accompanied by the marked increase of RII *in vitro* (Tortora et al., 1991; Yokozaki et al., 1993) and type II PKA activity in nude mice injected with cancer cells (Nesterova and Cho-Chung, 1995). However, in neuroblastoma cells, the down-regulation of RIα and type I PKA by antisense expression of RIα could not induce an increase of RII or type II PKA activity (Kim et al., 1997). In the present study, we observed only slight increase of RIIβ in RIα antisense-expressing cells. The difference in the changes of RIIβ levels seems to be due to the differences in the endogenous level of RIIβ and in the level of RIα decrease. As shown in the study with neuroblastoma cells, without basal level of RIIβ, decrease of RIα could not induce RIIβ expression (Kim et al., 1997). However, in ovarian cancer cells, only 30% decrease in RIα resulted in the increase of RIIβ. Thus, the endogenous PKA isozyme status may be important in the induction of RIIβ. In the studies using RIα antisense oligodeoxynucleotide, RIα mRNA and protein were eliminated (Tortora et al., 1991; Yokozaki et al., 1993), but in the case of antisense expression, RIα protein still remained (Fig. 4). Thus, it may be reasoned that the antisense expression of RIα in this study was not so effective as RIα antisense oligodeoxynucleotide.

We have shown in the present study that the modulation of PKA isozymes, i.e., up-regulation of RIIβ and down-regulation of RIα, can induce growth inhibition in ovarian cancer cells. Interestingly, decrease of RIα by antisense expression caused an increase of RIIβ and growth inhibition, suggesting that the intracellular level of RIα can affect that of RIIβ in ovarian cells. These results indicate that the modulation of PKA isozymes using cAMP analogues, antisense oligonucleotides, or gene transfer, could be applied to control the growth of ovarian cancer cells.

Acknowledgments

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References

Alley MC, Scudiero DA, Monks A, Hursey ML, Czerwinski MJ, Fine DL, Abbott BJ, Mayo JG, Shoemaker RH, and Boyd MR (1988) Feasibility of drug screening with panels of human tumor cell lines using a microculture tetrazolium assay. *Cancer Res* 48: 589-601.

Ally S, Clair T, Katsaros D, Tortora G, Yokozaki H, Finch RA, Avery TL, and Cho-Chung YS (1989) Inhibition of growth and modulation of gene expression in human lung carcinoma in athymic mice by site-selective 8-Cl-cyclic adenosine monophosphate. *Cancer Res* 49: 5650-5655.

Ally S, Tortora G, Clair T, Grieco D, Merlo G, Katsaros D, Ogreid D, Døskeland SO, Jahnsen T, and Cho-Chung YS (1988) 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.

Budillon A, Cereseto A, Kondrashin A, Nesterova M, Merlo G, Clair T, and Cho-Chung YS (1995) Point mutation of the autophosphorylation site or in the nuclear location signal causes protein kinase A RIIβ regulatory subunit to lose its ability to revert transformed fibroblasts. *Proc Natl Acad Sci USA* 92: 10634-10638.

Cho-Chung, YS (1990) Role of cyclic AMP receptor proteins in growth, differentiation, and suppression of malignancy: new approaches to therapy. *Cancer Res* 50: 7093-7100.

Clair T, Ally S, Tagliaferri P, Robins RK, and Cho-Chung YS (1987) Site-selective cAMP analogs induce nuclear translocation of the RII cAMP receptor protein in Ha-MuSV-tran-

sformed NIH/3T3 cells. *FEBS Lett* 224: 377-384.
Darbon J-M, Knecht M, Ranta T, Dufau ML, and Catt KJ (1984) Hormonal regulation of cyclic AMP-dependent protein kinase in cultured ovarian granulosa cells. *J Biol Chem* 259: 14778-14782.
DeAngelo AB, Schweppe JS, Jungmann JA, Huber P, and Eppenberger U (1975) Ovarian cyclic adenosine monophosphate-dependent protein kinase activity: ontogeny and effect of gonadotropins. *Endocrinology* 97: 1509-1520.
Hunzicker-Dunn M (1982) Rat ovarian nuclear protein kinases. *Biochim Biophys Acta* 714: 395-406.
Katsaros D, Tortora G, Tagliaferri P, Clair T, Ally S, Neckers L, Robins RK, and Cho-Chung YS (1987) Site-selective cyclic AMP analogs provide a new approach in the control of cancer cell growth. *FEBS Lett* 223: 97-103.
Kim SN, Lee GR, Cho-Chung YS, Park SD, and Hong SH (1996) Overexpression of RIIβ regulatory subunit of protein kinase A induces growth inhibition and reverse-transformation in SK-N-SH human neuroblastoma cells. *Intl J Oncol* 8: 663-668.
Kim SN, Lee GR, Hwang ES, Lee J-H, Park SD, Cho-Chung YS, and Hong SH (1997) Type II protein kinase A up-regulation is sufficient to induce growth inhibition in SK-N-SH human neuroblastoma cells. *Biochem Biophys Res Commun* 232: 469-473.
Knecht M, Amsterdam A, and Catt K (1981) The regulatory role of cyclic AMP in hormone-induced granulosa cell differentiation. *J Biol Chem* 256: 10628-10633.
Levy FO, Øyen O, Sandberg M, Taskén K, Eskild W, Hansson V, and Jahnsen T (1988) Molecular cloning, complementary deoxyribonucleic acid structure and predicted full-length amino acid sequence of the hormone-inducible regulatory subunit of 3′-5′-cyclic adenosine monophosphate-dependent protein kinase from human testis. *Mol Endocrinol* 2: 1364-1373.
McGeady ML, Kerby S, Shankar V, Ciardiello F, Salomon DS, and Seidman M (1989) Infection with a TGF-α retroviral vector transforms normal mouse mammary epithelial cells but not normal rat fibroblasts. *Oncogene* 4: 1375-1382.
McKnight GS, Clegg CH, Uhler MD, Chrivia JC, Cadd GG, Correll LA, and Otten AD (1988) Analysis of the cAMP-dependent protein kinase system using molecular genetic approaches. *Recent Prog Horm Res* 44: 307-335.
Mednieks MI, Yokozaki H, Merlo GR, Tortora G, Clair T, Ally S, Tahara E, and Cho-Chung YS (1989) Site-selective 8-Cl-cAMP which causes growth inhibition and differentiation increases DNA (CRE)-binding activity in cancer cells. *FEBS Lett* 254: 83-88.
Nesterova M and Cho-Chung YS (1995) A single-injection protein kinase A-directed antisense treatment to inhibit tumour growth. *Nature Med* 1: 528-533.
Nesterova M, Yokozaki H, McDuffie E, and Cho-Chung YS (1996) Overexpression of RIIβ regulatory subunit of protein kinase A in human colon carcinoma cell induces growth arrest and phenotypic changes that are abolished by site-directed mutation of RIIβ. *Eur J Biochem* 235: 486-494.
Otten AD and McKnight GS (1989) Overexpression of the type II regulatory subunit of the cAMP-dependent protein kinase eliminates the type I holoenzyme in mouse cells. *J Biol Chem* 264: 20255-20260.
Ratoosh SL, Lifka J, Hedin L, Jahnsen T, and Richards J (1987) Hormonal regulation of the synthesis and mRNA content of the regulatory subunit of cyclic AMP-dependent protein kinase type II in cultured rat ovarian granulosa cells. *J Biol Chem* 262: 7306-7313.
Rohlff C, Clair T, and Cho-Chung YS (1993) 8-Cl-cAMP induces truncation and down-regulation of the RIα subunit and up-regulation of the RIIβ subunit of cAMP-dependent protein kinase leading to type II holoenzyme-dependent growth inhibition and differentiation of HL-60 leukemia cells. *J Biol Chem* 268: 5774-5782.
Sandberg M, Taskén K, Øyen O, Hansson V, and Jahnsen T (1987) Molecular cloning, cDNA structure and deduced amino acid sequence for a type I regulatory subunit of cAMP-dependent protein kinase from human testis. *Biochem Biophys Res Commun* 149: 939-945.
Seo J, Park W, Kim JS, Hwang ES, Lee J-H, and Hong SH (1995) Endogenous gene expression of p53 and regulatory subunits of cyclic AMP-dependent protein kinase in ovarian cancer cells. *Korean J Zool* 3: 204-211.
Steinberg RA and Agard DA (1981) Turnover of regulatory subunit of cyclic AMP- dependent protein kinase in S49 mouse lymphoma cells. *J Biol Chem* 256: 10731-10734.
Sutherland EW, and Rall TW (1957) Properties of an adenine ribonucleotide produced with cellular particles, adenosinetriphosphate, magnesium, and adrenaline or glucagon. *J Am Chem* 79: 3608.
Tortora G, Clair T, Katsaros D, Ally S, Colamonici O, Neckers LM, Tagliaferri P, Jahnsen T, Robins RK, and Cho-Chung YS (1989a) Induction of megakaryocytic differentiation and modulation of protein kinase gene expression by site-selective cAMP analogs in K-562 human leukemic cells. *Proc Natl Acad Sci USA* 86: 2849-2852.
Tortora G, Ciardiello F, Ally S, Clair T, Salomon DS, and Cho-Chung YS (1989b) Site-selective 8-chloroadenosine 3′,5′-cyclic monophosphate inhibits transformation and transforming growth factor α production in Ki-ras-transformed rat fibroblasts. *FEBS Lett* 242: 363-367.
Tortora G, Clair T, and Cho-Chung YS (1990a) An antisense oligodeoxynucleotide targeted against the type II regulatory subunit mRNA of protein kinase inhibits cAMP-induced differentiation in HL-60 leukemia cells without affecting phorbol ester effects. *Proc Natl Acad Sci USA* 87: 705-708.
Tortora G and Cho-Chung YS (1990b) Type II regulatory subunit of protein kinase restores cAMP-dependent transcription in a cAMP-unresponsive cell line. *J Biol Chem* 265: 18067-18070.
Tortora G, Yokozaki H, Pepe S, Clair T, and Cho-Chung YS (1991) Differentiation of HL-60 leukemia by type I regulatory subunit antisense oligodeoxynucleotide of cAMP-dependent protein kinase. *Proc Natl Acad Sci USA* 88: 2011-2015.
Tortora G, Pepe S, Bianco C, Baldassarre G, Budillon A, Clair T, Cho-Chung YS, Bianco AR, and Ciardiello F (1994) The RIα subunit of protein kinase A controls serum dependency and entry into cell cycle of human mammary epithelial cells. *Oncogene* 9: 3233-3240.
Yokozaki H, Budillon A, Tortora G, Meissner S, Beaucage SL, Miki K, and Cho-Chung YS (1993) An antisense oligodeoxynucleotide that depletes RIα subunit of cyclic AMP-dependent protein kinase induces growth inhibition in human cancer cells. *Cancer Res* 53: 868-872.

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