Cyclic AMP-mediated growth suppression and MAPK phosphorylation in thyroid papillary carcinoma cells

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Abstract. The main regulating systems of thyroid growth are the mitogen-activated protein kinase (MAPK) signaling pathway and the cAMP signaling pathway. Thyroid papillary carcinoma frequently involves mutations in *BRAF* or *RET/PTC* without overlap, which are expected to constitutively activate MAPK signaling. On the other hand, it has been reported that cAMP signaling acts in an inhibitory manner on the proliferation of papillary carcinoma cell lines, although the cAMP pathway physiologically promotes the proliferation of normal follicular cells as well as hormonogenesis. The effect of cAMP on proliferation is attributed to crosstalk with MAPK signaling. However, this phenomenon has not been clearly established in papillary carcinoma with *BRAF* or *RET/PTC* mutations. In order to elucidate whether activated cAMP signaling inhibits cell proliferation and affects MAPK signaling in papillary carcinoma, we performed *in vitro* experiments using two representative cell lines, K1 and TPC-1, which have a *BRAF* and an *RET/PTC* mutation, respectively. Elevated cAMP caused by an adenylate cyclase activator suppressed the proliferation of both K1 and TPC-1 cells. Examining the crosstalk between cAMP and MAPK signaling, K1 and TPC-1 cells showed opposite responses to cAMP activation. These responses were blocked by an inhibitor of the cAMP-dependent protein kinase (PKA). In K1 cells, B-Raf might predominate over Raf-1, and the elevated cAMP is thought to promote MAPK phosphorylation through the PKA-mediated activation of Rap1. On the other hand, in TPC-1 cells Raf-1 might predominate and could be inhibited by activated Rap1, resulting in the suppression of MAPK phosphorylation. In conclusion, the proliferation of both papillary carcinoma cell types was significantly suppressed by cAMP signaling, regardless of whether MAPK signaling was activated or inactivated by the PKA-mediated cAMP signaling pathway. There could, however, be other mechanisms by which cAMP signaling inhibits the growth of papillary carcinoma cells.

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Introduction

The main regulators of thyroid growth are thyroid-stimulating hormone (TSH) and growth factors. Developmentally and physiologically, TSH promotes follicular proliferation as well as hormone synthesis by binding to the thyroid-stimulating hormone receptor (TSHR) and activating the cAMP-mediated signaling pathway. Growth factors act through tyrosine kinase receptors and specifically activate signaling pathways such as RAS/mitogen-activated protein kinase (MAPK) (1). It is generally known that the cascade mediated by the tyrosine kinase receptor plays an important role in tumor progression. The constitutive activation of molecules upstream of the MAPK signaling pathway is sufficient for tumorigenesis (2).

The role of TSH and the cAMP signaling pathway in the proliferation of thyroid tumor cells is not well understood. Previous *in vivo* studies reported that well-differentiated thyroid carcinoma expressed TSHR, and there is a clear inverse relationship between the expression of TSHR or adenylate cyclase activity and the degree of differentiation of thyroid carcinoma (3,4). *In vitro* studies have revealed that the stimulation of TSHR is mediated through a heterotrimeric Gs protein, which activates the adenylate cyclase-cAMP-cAMP-dependent protein kinase (PKA) cascade (5). cAMP could have either mitogenic or anti-mitogenic effects depending on the cell type (6). In thyroid follicular cells, cAMP stimulates proliferation, although the participation of other factors, such as IGF-1, EGF or those present in serum, are required for cAMP to display full mitogenic activity (7,8). The effect of cAMP on thyroid carcinoma growth is somewhat controversial. In papillary carcinoma, it has been shown by using many culture cell lines (HTC transfected TSHR, hPTC, NP, BHP2-7, BHP7-13, BHP10-3, BHP18-21, BHP17-10 and K1) that cAMP is anti-mitogenic and acts as a growth inhibitor in a dose-dependent manner (9-13). On the other hand, in a follicular carcinoma cell line (FTC 133), it was reported that cell proliferation was promoted by low-dose TSH treatment and inhibited by high-dose TSH treatment (14).

Recent years have been marked by dramatic developments in the understanding of the molecular basis of thyroid carcinogenesis. Mutations of *BRAF* and *RET/PTC* in papillary carcinoma, expected to lead to the activation of the MAPK signaling pathway, are found in more than 70% of cases with practically no overlap. This provides genetic evidence that constitutive signaling along the RET-RAS-RAF-MAPK pathway is key to the development of papillary carcinoma (15).

One of the possible mechanisms for the anti-mitogenic effect of cAMP in papillary carcinoma is an attenuation of the MAPK signaling pathway. Although it is widely accepted that the cell type-specific effects of cAMP on proliferation are largely attributed to crosstalk between cAMP and the MAPK signaling pathway (16,17), the crosstalk has not yet been clearly established in papillary carcinoma. In addition, whether or not the *BRAF* and *RET/PTC* mutations found in papillary carcinoma influence this crosstalk has not been investigated. If the cAMP signaling pathway inhibits MAPK phosphorylation in papillary carcinoma, this pathway may be one of the mechanisms which can explain the slow proliferation of papillary carcinoma.

In the present study, we hypothesized that the activation of cAMP signaling acts in a suppressive manner on the cell proliferation of papillary carcinoma. With the *BRAF* and *RET/PTC* mutations, we performed *in vitro* experiments using two representative culture cells of thyroid papillary carcinoma in order to elucidate whether the cAMP signaling pathway inhibits cell proliferation and affects the MAPK signaling pathway.

Materials and methods

Cell lines and culture conditions. We used two established cell lines of thyroid papillary carcinoma: K1 cells, which possess the activating point mutation of the *BRAF* gene resulting in V600E, and TPC-1 cells, which have rearrangements of *RET/PTC* (18). We selected these two cell lines since they contained the two mutations representatively found in most *in vivo* papillary carcinomas. Neither of the cell lines expressed TSHR, which was confirmed by RT-PCR (data not shown). Both cell types were routinely maintained in DMEM/HamF-12 (1:1) supplemented with 10% FBS, 1% ITS+1 (Sigma) and antibiotics at 37˚C under 5% CO_2. For all experiments, cells were initially cultivated in regular growth medium for at least 48 h. As appropriate for individual experiments, cells were then shifted to medium containing 2.5% FBS without ITS+1 for 24-48 h prior to analysis.

MTT assay. Cells were seeded at a density of $2x10^3$ cells/well into 96-well plates and incubated for 48 h under 0, 1, 5 and 10 ng/ml IGF-1 (PeproTech, Rocky Hill, NJ) stimulation with or without 10^{-5} M forskolin (Biomol, Plymouth Meeting, PA), which is an activator of adenylate cyclase. At the indicated time points, MTT (500 μg/ml) was added to each well, and the cells were incubated for 4 h at 37˚C. The cells were then lysed and solubilized with extraction buffer (up to 20% SDS dissolved in 50% dimethylsulfomaide solution) for 2 h at 37˚C with gentle shaking. Absorbance was measured at 750 nm with a microplate reader (BioRad Model 680).

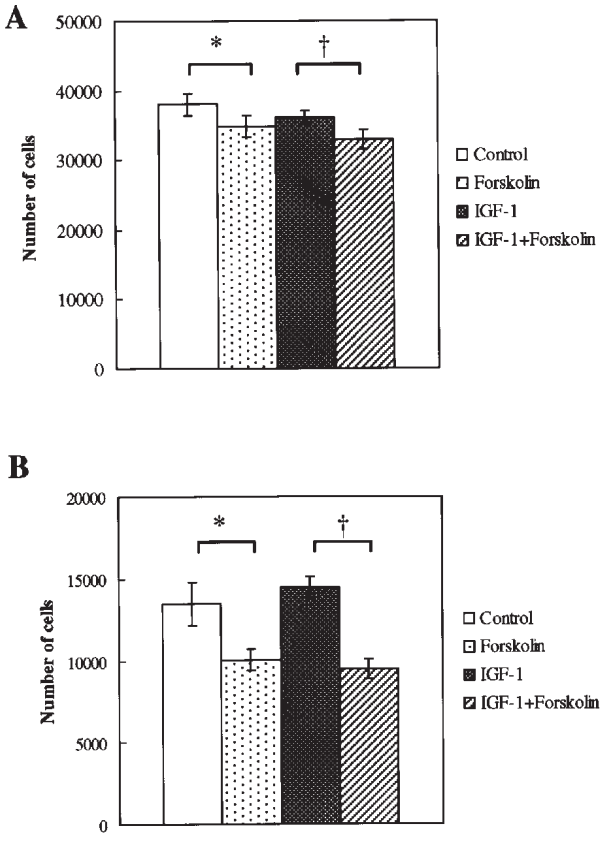

Figure 1. Effect of IGF-1 and forskolin on the proliferation of K1 and TPC-1 cells. MTT assays were performed on K1 (A) and TPC-1 (B) cells. Both cell lines ($2x10^3$ cells/well) were incubated for 48 h under 1 ng/ml IGF-1 stimulation with or without 10^{-5} M forskolin treatment. Data are expressed as the mean ± SD of 8 replicates. *$p<0.05$ when compared with control values; †$p<0.05$ when compared with cells treated with IGF-1.

Statistical analysis was performed using the Student's t-test, with $p<0.05$ considered to be statistically significant.

Western blot analysis. Cells were solubilized in lysis buffer [62.5 mM Tris/HCl (pH 6.8), 2% sodium dodecylsulfate (SDS), 10% glycerol, 50 mM DTT and 0.01% bromophenol blue] at 3, 5 and 10 min following incubation with 1 ng/ml IGF-1 with or without 10^{-5} M forskolin stimulation. In experiments using a selective PKA inhibitor, H-89 (Biomol), cells were pretreated with 50 μM H-89 for 5 min prior to stimulation. Total cell lysates (20-30 μg protein per lane) were separated by 12.5% SDS-polyacrylamide gel electrophoresis (PAGE) and transferred to nitrocellulose membranes (Schleicher and Schuell, Keene, NH). The resulting blots were blocked for 1 h at room temperature with 5% skim milk dissolved in phosphate-buffered saline (PBS) and then probed with antibodies anti-p44/42 MAPK and anti-phospho-MAPK (Cell Signaling Technology, Beverly, MA) according to the manufacturer's recommendations. After five washes in PBS/Tween, the membranes were incubated for 30 min with peroxidase-labeled secondary antibody (Amersham Biosciences, Buckinghamshire, UK) diluted 1:5000 at room temperature. After three additional washes, immunoblots were developed using the enhanced chemiluminescence detection system (Western Lightning, PerkinElmer Life Sciences).

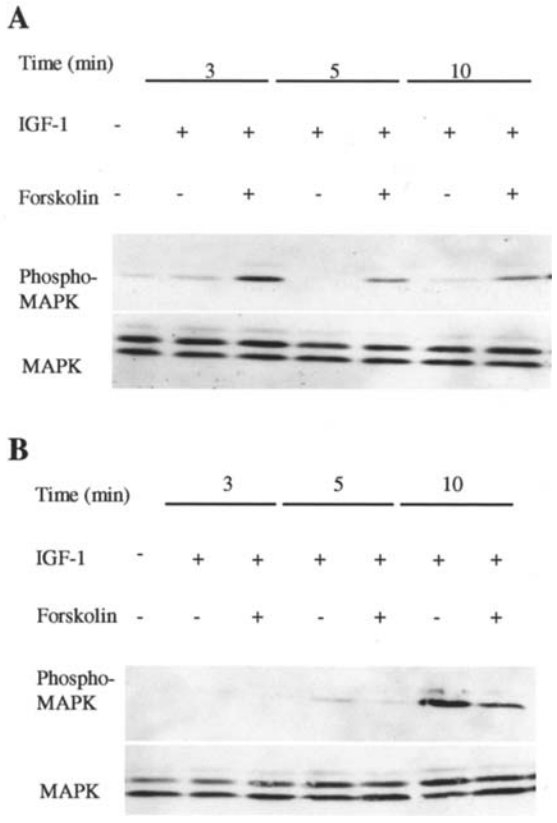

Figure 2. Effect of IGF-1 and forskolin on MAPK activation under IGF-1 stimulation. Cells were treated with 1 ng/ml IGF-1 with or without 10^{-5} M forskolin. The level of MAPK phosphorylation was examined at different time points in K1 (A) and TPC-1 (B) cells by Western blotting.

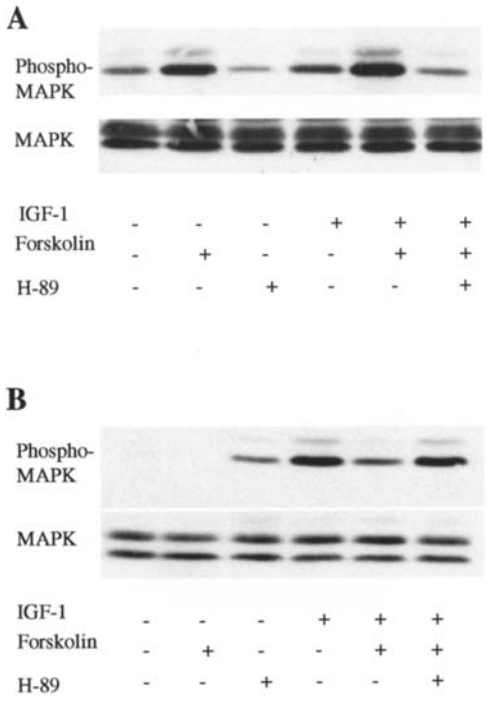

Figure 3. Effect of H-89 on IGF-1- or forskolin-induced MAPK phosphorylation. Analysis was performed at the time point when apparent differences in MAPK phosphorylation levels between IGF-1 and IGF-1 as well as forskolin treatment were detected in Fig. 2 [K1 cells (A) 3 min and TPC-1 cells (B) 10 min after stimulation].

Results

Suppressed proliferation by adenylate cyclase activator. The effect of forskolin, an activator of adenylate cyclase that rapidly elevates intracellular cAMP level, on cellular proliferation was assayed by means of MTT. Both cell lines incubated in forskolin exhibited a significant reduction in cell number compared with the control (Fig. 1). In addition, to clarify the effect of forskolin on IGF-1-induced proliferation, cells were treated with IGF-1 (1, 5 and 10 ng/ml) alone or in the presence of forskolin. However, IGF-1 (1 ng/ ml) by itself did not significantly influence the growth of either of the cell lines compared with the control (Fig. 1), neither did stimulation with 5 and 10 ng/ml IGF-1 show a significant difference in growth (data not shown). However, under IGF-1 stimulation (1 ng/ml in K1 cells; 1 and 5 ng/ml in TPC-1 cells), the inhibitory effect of forskolin was also significant compared to the control (Fig. 1).

Effects of adenylate cyclase activator on MAPK phosphorylation. MAPK phosphorylation was measured to determine whether IGF-1 induced MAPK and to evaluate the effect of forskolin-induced elevations in intracellular cAMP levels on MAPK activity. Therefore, we analyzed MAPK phosphorylation stimulated by IGF-1 in the presence or absence of forskolin by Western blot analysis. In K1 cells, IGF-1 stimulation alone slightly increased phospho-MAPK expression, and IGF-1 with forskolin appreciably increased its expression in the absence of a significant change in total MAPK, with a maximal effect being detected 3 min after addition (Fig. 2A). In TPC-1 cells, forskolin blocked IGF-1-induced MAPK phosphorylation, detectable 5-10 min after addition (Fig. 2B).

The role of PKA in crosstalk between the cAMP and MAPK signaling pathways. To investigate the involvement of PKA in crosstalk between the cAMP and MAPK signaling pathways, we examined the effect of H-89, a selective inhibitor of PKA, on MAPK phosphorylation. H-89 alone slightly reduced basal MAPK phosphorylation in K1 cells (Fig. 3A). On the other hand, H-89 increased basal MAPK phosphorylation in TPC-1 cells (Fig. 3B). Furthermore, we evaluated whether H-89 affected forskolin-induced activation or inhibition of the MAPK signaling pathway under IGF-1 stimulation. H-89 was able to reverse the effect of forskolin in both cell lines (Fig. 3). These results proved that the crosstalk between the cAMP and MAPK signaling pathways was mediated by PKA, regardless of whether MAPK was phosphorylated or not.

Discussion

In this study, we used forskolin to activate adenylate cyclase and to elevate intracytoplasmic cAMP because the available cell lines of papillary carcinoma did not express TSHR. We demonstrated that activated cAMP signaling suppresses the proliferation of K1 and TPC-1 cells derived from thyroid papillary carcinoma, which is quite compatible with previous studies (9-13). It seems reasonable to suppose that elevated cAMP by itself plays a key role in the inhibitory effect of

TSH-cAMP signaling on the cell proliferation of papillary carcinoma. However, a knowledge of far more complex mechanisms could be required to understand the alternative effects of cAMP; i.e., its mitogenetic effect on normal follicular cells and anti-mitogenetic effect on papillary carcinoma cells.

Examining the crosstalk between the cAMP and MAPK signaling pathways and K1 and TPC-1 cells with mutations of *BRAF* and *RET/PTC*, respectively, showed opposite responses to adenylate cyclase activation. These responses were blocked by an inhibitor of cAMP-dependent protein kinase (PKA). The activated cAMP signaling, producing elevated intracellular cAMP, activated PKA and prompted MAPK phosphorylation in K1 cells, although it resulted in growth inhibition. On the contrary, PKA-mediated cAMP signaling suppressed MAPK phosphorylation in TPC-1 cells. These findings indicate that MAPK is not directly phosphorylated by PKA. The MAPK signaling pathway is tightly regulated by crosstalk with other signaling pathways, and the cAMP signaling pathway is one of the best-characterized signals by which the activation of MAPK is regulated. cAMP inhibits the growth of many types of cells with little B-Raf expression, such as fibroblasts (19,20), smooth muscle cells (21) and adipocytes (22), by blocking the binding of Raf-1 to Ras (23) and thus suppressing the MAPK pathway. On the contrary, in endocrine cells, such as pheochromocytoma PC12 cells, cAMP induces phosphorylation of MAPK through PKA-mediated activation of the Ras-related small G protein, Rap1 (24). Activated Rap1 is both a selective activator of B-Raf and an inhibitor of Raf-1. Thus, the net effect on MAPK activation depends on the predominance of either B-Raf or Raf-1 (16,17,24-28). Our results can also be explained by the widely-accepted model mentioned above. Namely, B-Raf might predominate over the inhibition of Raf-1 in K1 cells with mutated *BRAF*, and MAPK could be phosphorylated through the elevated cAMP- and PKA-mediated activation of Rap1. On the other hand, Raf-1 might predominate and could be inhibited by activated Rap1 in TPC-1 cells, resulting in the suppression of MAPK phosphorylation.

Because the proliferation of both K1 and TPC-1 cells was suppressed regardless of the phosphorylation state of MAPK, there may be another mechanism, besides a MAPK-dependent one, by which cAMP inhibits the growth of papillary carcinoma cells. Several other mechanisms have been proposed to explain the antiproliferative effects of cAMP on various other cells and tissues, including increasing the amount of p21 (29) and decreasing the expression level of cyclin D1 (30). These mechanisms are probably related to the induction of immediate early genes such as *c-jun*, which is known to transactivate the cyclin D1 promoter and is regulated by TSH (31,32). In thyroid follicular cells, the catalytic unit of PKA (PKA-C) is activated by cAMP to translocate to the nuclear compartment from the cytoplasm. PKA-C phosphorylates the cAMP response element binding protein (CREB), which has several splicing variants with alternative functions to either activate (33,34) or inactivate (35-37) the transcription of many cAMP-responsive genes that regulate cell division, such as *c-jun*, *c-fos* and *c-myc*, within 15-30 min of TSH induction (38). It is noteworthy that, contrary to the results of this study, our previous report (13) demonstrated forskolin-induced MAPK suppression in K1 cells at different time points, 30-60 min follwing IGF-1 stimulation under the 48-h pretreatment with forskolin. This finding may be related to the regulation by alternative splicing of CREB, corresponding to the immediate response of the early inducible genes of DNA synthesis.

In conclusion, we demonstrated that an adenylate cyclase activator inhibits the proliferation of two thyroid papillary carcinoma cell lines, K1 and TPC-1, which have the representative mutations *BRAF* and *RET/PTC*, respectively. The proliferation of both cell types was significantly suppressed by cAMP signaling, regardless of whether MAPK signaling was activated or inactivated by the PKA-mediated cAMP signaling pathway.

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References

1. Marshall CJ: Specificity of receptor tyrosine kinase signaling: transient versus sustained extracellular signal-regulated kinase activation. Cell 80: 179-185, 1995.
2. Mansour SJ, Matten WT, Hermann AS, Candia JM, Rong S, Fukasawa K, van de Woude GF and Ahn NG: Transformation of mammalian cells by constitutively active MAP kinase kinase. Science 265: 966-970, 1994.
3. Abe Y, Ichikawa Y, Muraki T, Ito K and Homma M: Thyrotropin (TSH) receptor and adenylate cyclase activity in human thyroid tumors: absence of high affinity receptor and loss of TSH responsiveness in undifferentiated thyroid carcinoma. J Clin Endocrinol Metab 52: 23-28, 1981.
4. Sheils OM and Sweeney EC: TSH receptor status of thyroid neoplasms - TaqMan RT-PCR analysis of archival material. J Pathol 188: 87-92, 1999.
5. Vassart G and Dumont JE: The thyrotropin receptor and the regulation of thyrocyte function and growth. Endocr Rev 13: 596-611, 1992.
6. Iyengar R: Gating by cyclic AMP: expanded role for an old signaling pathway. Science 271: 461-463, 1996.
7. Medina DL and Santisteban P: Thyrotropin-dependent proliferation of *in vitro* rat thyroid cell systems. Eur J Endocrinol 143: 161-178, 2000.
8. Kimura T, van Keymeulen A, Golstein J, Fusco A, Dumont JE and Roger PP: Regulation of thyroid cell proliferation by TSH and other factors: a critical evaluation of *in vitro* models. Endocr Rev 22: 631-656, 2001.
9. Kimura H, Yamashita S, Namba H, Tominaga T, Tsuruta M, Yokoyama N, Izumi M and Nagataki S: Interleukin-1 inhibits human thyroid carcinoma cell growth. J Clin Endocrinol Metab 75: 596-602, 1992.
10. Derwahl M, Kuemmel M, Goretzki P, Schatz H and Broecker M: Expression of the human TSH receptor in a human thyroid carcinoma cell line that lacks an endogenous TSH receptor: growth inhibition by cAMP. Biochem Biophys Res Commun 191: 1131-1138, 1993.
11. Hishinuma A, Yamanaka T, Kasai K, So S, Bamba N and Shimoda SI: Growth regulation of the human papillary thyroid cancer cell line by protein tyrosine kinase and cAMP-dependent protein kinase. Endocr J 41: 399-407, 1994.
12. Ohta K, Pang XP, Berg L and Hershman JM: Growth inhibition of new human thyroid carcinoma cell lines by activation of adenylate cyclase through the beta-adrenergic receptor. J Clin Endocrinol Metab 82: 2633-2638, 1997.
13. Yano Y, Kamma H, Matsumoto H, Fujiwara M, Bando H, Hara H, Yashiro T, Ueno E, Ito K and Uchida K: Growth suppression of thyroid cancer cells by adenylcyclase activator. Oncol Rep 18: 441-445, 2007.
14. Hoelting T, Tezelman S, Siperstein AE, Duh QY and Clark OH: Thyrotropin stimulates invasion and growth of follicular thyroid cancer cells via PKC- rather than PKA-activation. Biochem Biophys Res Commun 195: 1230-1236, 1993.

15. Adeniran AJ, Zhu Z, Gandhi M, Steward DL, Fidler JP, Giordano TJ, Biddinger PW and Nikiforov YE: Correlation between genetic alterations and microscopic features, clinical manifestations, and prognostic characteristics of thyroid papillary carcinomas. Am J Surg Pathol 30: 216-222, 2006.
16. Dumaz N and Marais R: Integrating signals between cAMP and the RAS/RAF/MEK/ERK signalling pathways. Based on the anniversary prize of the Gesellschaft fur Biochemie und Molekularbiologie Lecture delivered on 5 July 2003 at the special FEBS meeting in Brussels. FEBS J 272: 3491-3504, 2005.
17. Stork PJ and Schmitt JM: Crosstalk between cAMP and MAP kinase signaling in the regulation of cell proliferation. Trends Cell Biol 12: 258-266, 2002.
18. Soares P, Trovisco V, Rocha AS, Lima J, Castro P, Preto A, Maximo V, Botelho T, Seruca R and Sobrinho-Simoes M: BRAF mutations and RET/PTC rearrangements are alternative events in the etiopathogenesis of PTC. Oncogene 22: 4578-4580, 2003.
19. Hordijk PL, Verlaan I, Jalink K, van Corven EJ and Moolenaar WH: cAMP abrogates the p21ras-mitogen-activated protein kinase pathway in fibroblasts. J Biol Chem 269: 3534-3538, 1994.
20. Burgering BM, Pronk GJ, van Weeren PC, Chardin P and Bos JL: cAMP antagonizes p21ras-directed activation of extracellular signal-regulated kinase 2 and phosphorylation of mSos nucleotide exchange factor. EMBO J 12: 4211-4220, 1993.
21. Graves LM, Bornfeldt KE, Raines EW, Potts BC, Macdonald SG, Ross R and Krebs EG: Protein kinase A antagonizes platelet-derived growth factor-induced signaling by mitogen-activated protein kinase in human arterial smooth muscle cells. Proc Natl Acad Sci USA 90: 10300-10304, 1993.
22. Sevetson BR, Kong X and Lawrence JC Jr: Increasing cAMP attenuates activation of mitogen-activated protein kinase. Proc Natl Acad Sci USA 90: 10305-10309, 1993.
23. Wu J, Dent P, Jelinek T, Wolfman A, Weber MJ and Sturgill TW: Inhibition of the EGF-activated MAP kinase signaling pathway by adenosine 3',5'-monophosphate. Science 262: 1065-1069, 1993.
24. Vossler MR, Yao H, York RD, Pan MG, Rim CS and Stork PJ: cAMP activates MAP kinase and Elk-1 through a B-Raf- and Rap1-dependent pathway. Cell 89: 73-82, 1997.
25. Cook SJ and McCormick F: Inhibition by cAMP of Ras-dependent activation of Raf. Science 262: 1069-1072, 1993.
26. Mischak H, Seitz T, Janosch P, Eulitz M, Steen H, Schellerer M, Philipp A and Kolch W: Negative regulation of Raf-1 by phosphorylation of serine 621. Mol Cell Biol 16: 5409-5418, 1996.
27. Dugan LL, Kim JS, Zhang Y, Bart RD, Sun Y, Holtzman DM and Gutmann DH: Differential effects of cAMP in neurons and astrocytes. Role of B-raf. J Biol Chem 274: 25842-25848, 1999.
28. Schmitt JM and Stork PJ: Cyclic AMP-mediated inhibition of cell growth requires the small G protein Rap1. Mol Cell Biol 21: 3671-3683, 2001.
29. Lee TH, Chuang LY and Hung WC: Tamoxifen induces p21WAF1 and p27KIP1 expression in estrogen receptor-negative lung cancer cells. Oncogene 18: 4269-4274, 1999.
30. L'Allemain G, Lavoie JN, Rivard N, Baldin V and Pouyssegur J: Cyclin D1 expression is a major target of the cAMP-induced inhibition of cell cycle entry in fibroblasts. Oncogene 14: 1981-1990, 1997.
31. Heinrich R and Kraiem Z: The protein kinase A pathway inhibits c-jun and c-fos protooncogene expression induced by the protein kinase C and tyrosine kinase pathways in cultured human thyroid follicles. J Clin Endocrinol Metab 82: 1839-1844, 1997.
32. Bakiri L, Lallemand D, Bossy-Wetzel E and Yaniv M: Cell cycle-dependent variations in c-Jun and JunB phosphorylation: a role in the control of cyclin D1 expression. EMBO J 19: 2056-2068, 2000.
33. Hai TW, Liu F, Coukos WJ and Green MR: Transcription factor ATF cDNA clones: an extensive family of leucine zipper proteins able to selectively form DNA-binding heterodimers. Genes Dev 3: 2083-2090, 1989.
34. Rehfuss RP, Walton KM, Loriaux MM and Goodman RH: The cAMP-regulated enhancer-binding protein ATF-1 activates transcription in response to cAMP-dependent protein kinase A. J Biol Chem 266: 18431-18434, 1991.
35. Foulkes NS, Borrelli E and Sassone-Corsi P: CREM gene: use of alternative DNA-binding domains generates multiple antagonists of cAMP-induced transcription. Cell 64: 739-749, 1991.
36. Cowell IG, Skinner A and Hurst HC: Transcriptional repression by a novel member of the bZIP family of transcription factors. Mol Cell Biol 12: 3070-3077, 1992.
37. Karpinski BA, Morle GD, Huggenvik J, Uhler MD and Leiden JM: Molecular cloning of human CREB-2: an ATF/CREB transcription factor that can negatively regulate transcription from the cAMP response element. Proc Natl Acad Sci USA 89: 4820-4824, 1992.
38. Hagiwara M, Brindle P, Harootunian A, Armstrong R, Rivier J, Vale W, Tsien R and Montminy MR: Coupling of hormonal stimulation and transcription via the cyclic AMP-responsive factor CREB is rate limited by nuclear entry of protein kinase A. Mol Cell Biol 13: 4852-4859, 1993.