Adenosine Triphosphate Activates Mitogen-Activated Protein Kinase in Pre-Neoplastic and Neoplastic Ovarian Surface Epithelial Cells

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ABSTRACT

To investigate the role of ATP in ovarian tumorigenesis, the present study examined the expression of the P2U purinoceptor (P2U-R) and effect of ATP on growth stimulation in pre-neoplastic and neoplastic ovarian surface epithelial (OSE) cells. The immortalized OSE (IOSE) cell lines, including IOSE-29 (pre-neoplastic), IOSE-29EC (neoplastic), and OVCA-3 (ovarian adenocarcinoma cell line) were used. Our results indicated that P2U-R mRNA was expressed and that ATP exerted a growth-stimulatory effect in IOSE-29, IOSE-29EC, and OVCA-3. To investigate the mechanism of the growth-stimulatory effect, the activation of mitogen-activated protein kinases (MAPKs) by ATP was examined. Treatment with ATP resulted in MAPK activation in IOSE-29 and IOSE-29EC cells, whereas the stimulatory effect of ATP in cellular proliferation and MAPK activation was completely abolished in the presence of PD98059 (an MAPK/ERK kinase inhibitor) and staurosporin (a protein kinase C inhibitor), suggesting that the growth stimulatory effect of ATP is mediated via protein kinase C-dependent MAPK activation in pre-neoplastic and neoplastic OSE cells. In a time-dependent study, ATP significantly increased MAPK activity at 5–20 min in IOSE-29 cells. Activated MAPK declined to control levels after 20 min in these cells. Treatment with ATP significantly induced MAPK activity after 5 min and was sustained for 60 min in IOSE-29EC cells. In addition, treatment with ATP resulted in substantial phosphorylation of Elk-1, the Ets family transcriptional factor, confirming that ATP action is mediated by activation of MAPK. In conclusion, we have demonstrated that P2U-R was expressed and that ATP induced growth stimulation in IOSE and OVCA-3 cells. Furthermore, treatment with ATP resulted in the activation of an MAPK cascade and phosphorylation of Elk-1 in IOSE-29 and IOSE-29EC cells. These results suggest that the MAPK cascade may be involved in growth stimulation in response to ATP in pre-neoplastic and neoplastic OSE cells.

INTRODUCTION

Extracellular ATP has been suggested to play a role in cellular proliferation and intracellular calcium concentration in ovarian cancer cells [1–3]. ATP is physiologically coreleased with neurotransmitter granules from nerve endings by exocytosis [4]. ATP activates hydrolysis of phosphoinositides, thereby generating diacylglycerol and inositol 1,4,5-trisphosphate, which stimulate protein kinase C (PKC) and cytosolic calcium mobilization, respectively [5, 6] after binding to heterotrimeric G protein-coupled P2 purinceptors. These results suggest that ATP may participate in various types of physiological responses, including secretion, membrane potential, cell proliferation, platelet aggregation, neurotransmission, cardiac function, and muscle contraction [6, 7]. Expression of ATP receptors, regulation of cell growth by ATP, and cellular functions of ATP have been studied in various ovarian [1–3] and breast cancer cells [8, 9].

Ovarian tumors appear to arise mainly from the ovarian surface epithelium (OSE), which is a simple squamous-to-cuboidal mesothelium that covers the ovary. The precise mechanism of ovarian tumorigenesis has not been elucidated [10]. Repeated ovulation and the process of healing ruptured OSE have been suggested to contribute to neoplastic transformation of OSE [11, 12]. Recently, the immortalized OSE (IOSE) cell lines IOSE-29 (pre-neoplastic) and IOSE-29EC (neoplastic) were directly generated from normal OSE by transfection with simian virus 40 (SV40)-large T antigen [13, 14]. IOSE-29EC cells were found to be anchorage-independent and formed transplantable, invasive subcutaneous and intraperitoneal adenocarcinomas in severely compromised immunodeficient mice, whereas the IOSE-29 line was nontumorigenic. This experimental culture model provides a unique system in which to examine the influences of contributing factors of OSE at progressive stages of neoplastic transformation. It has been demonstrated that extracellular ATP resulted in growth stimulation or inhibition and calcium mobilization in P2 purinoceptor-positive ovarian cancer cell lines, suggesting that ATP may act as an extracellular messenger in regulating ovarian cell proliferation via its specific receptors [1–3]. In previous reports we demonstrated that ATP may play an important role in the regulation of its receptors, induction of cytosolic calcium oscillations, activation of mitogen-activated protein kinases (MAPKs) and down-regulation of gonadotropic action in human granulosa-luteal cells [15–18]. Despite these findings, the precise molecular mechanism of ATP in terms of growth stimulation and intracellular signaling in ovarian cancer remains unknown. Considering the ovary is a well-innervated organ, it is tempting to speculate that coreleased ATP from nerve endings may play a role in regulating cellular proliferation and response in normal or neoplastic OSE cells if these cells express ATP receptors.
MAPKs are a group of serine/threonine kinases that are activated in response to a diverse array of extracellular stimuli and mediate signal transduction from the cell surface to the nucleus [19, 20]. These MAPKs are divided into three subgroups: extracellular signal-regulated kinases (ERKs), Jun N-terminal kinases/stress-activated protein kinases (JNKs/ SAPKs) and p38. It is well known that the MAPK cascade is activated by two distinct classes of cell surface receptors: receptor tyrosine kinases (RTKs) and heterotrimeric G protein-coupled receptors, which are composed of seven-transmembrane domains [20–24]. The signals transmitted through this cascade lead to activation of a set of molecules that regulate cell growth, division, and differentiation. The most extensively studied members of the cascade are ERK1 (p44 MAPK) and ERK2 (p42 MAPK). In addition, it has been demonstrated that MAPKs are regulated by cisplatin [25], paclitaxel [26], endothelin-1 [27], and GnRH [28] in ovarian cancer cells. Little is known about the molecular events that mediate ATP actions in neoplastic ovarian cells. Considering that ATP plays a role in the regulation of cell growth and calcium mobilization in ovarian cancer cells [1–3], we sought to investigate the effect of ATP in pre-neoplastic and neoplastic OSE cells including IOSE and OVCAR-3 cell lines. Experiments in the present study were designed to examine 1) the expression of P2U-R at the mRNA level in pre-neoplastic and neoplastic OSE cells including IOSE and OVCAR-3 cell lines. Experiments in the present study were designed to examine 1) the expression of P2U-R at the mRNA level in pre-neoplastic and neoplastic OSE cells, 2) the proliferative effect of ATP in these cells, and 3) the effect of ATP on MAPK activation.

MATERIALS AND METHODS

Materials

ATP, staurosporin, and PKC inhibitor were obtained from Sigma-Aldrich (St. Louis, MO). PD98059, an MAPK/ERK kinase (MEK) inhibitor, was purchased from New England Biolabs Inc. (Beverly, MA). Staurosporin and PD98059 were dissolved in dimethyl sulfoxide (DMSO) as suggested by the manufacturers.

Cell Culture

The nonmutagenic SV40 Tag-immortalized OSE-derived line (IOSE-29) and tumorigenic IOSE-29EC cells were cultured as previously described [13, 14] in medium 199:MCDB 105 (Sigma-Aldrich) containing 10% fetal bovine serum (FBS; Hyclone Laboratories Ltd., Logan, UT) and 100 U/ml penicillin G and 100 μg/ml streptomycin (Life Technologies Inc., Rockville, MD) in a humidified atmosphere of 5% CO2/95% air, and first-strand cDNA synthesis kit (Amersham Pharmacia Biotech). P-MAPK levels were quantitated using a commercial phospho-specific antibody and visualized using an enhanced chemiluminescent system (Amer- sham Pharmacia Biotech). The membrane was immunoblotted using a mouse monoclonal antibody specific to the phosphorylated p44/p42 MAPK (P-MAPK, Thr202/Tyr204) (New England Biolabs) [31]. Alternatively, the membrane was probed with a rabbit polyclonal antibody for p44/42 MAPK (New England Biolabs), which detects total MAPK (T-MAPK, phosphorylation state independent) levels. After washing, the signals were detected with horseradish peroxidase-conjugated secondary antibody and visualized using an enhanced chemiluminescent system (Amer- sham Pharmacia Biotech). P-MAPK levels were quantitated using a computerized visual light densitometer (model 620, BioRad Laboratories) and normalized against the levels of T-MAPK per sample. MAPK activity was represented as an P-MAPK-T:MAPK ratio.

RNA Extraction and Reverse Transcriptase-Polymerase Chain Reaction

Total RNA was prepared from cultured cells using the RNeasy kit (Bio/Can Scientific, Mississauga, ON, Canada) according to the manufacturer’s suggested procedure. RNA integrity was confirmed by using agarose gel electrophoresis and ethidium bromide staining. The total RNA concentration was determined from spectrophotometric analysis at A260/280. Complementary DNA was synthesized from 2.5 μg of total RNA by reverse transcriptase (RT) at 37°C for 2 h using a first-strand cDNA synthesis kit (Amersham Pharmacia Biotech, Baie d’Urfe, QC, Canada). The synthesized cDNA was used as a template for polymerase chain reaction (PCR) amplification. PCR was carried out with denaturing for 1 min at 94°C, annealing for 35 sec at 55°C, extension for 90 sec at 72°C, and a final extension for 15 min at 72°C using a DNA thermal cycler (Perkin-Elmer, Norwalk, CT). The primers were designed to amplify P2U-R mRNA based on the published sequences of human P2U-R [15]. The primers for P2U-

In Vitro MAPK Assay

IOSE-29 and IOSE-29EC cells were serum starved for 4 h. The cells were then treated with ATP (100 μM) in the presence or absence of PD98059 (50 μM) or staurosporin (0.1 μM) in a time-dependent manner. After treatments, the cells were washed twice with ice-cold PBS and lysed in ice-cold RIPA buffer (150 mM NaCl, 1% Nonidet P-40, 0.5% deoxycholate, 0.1% SDS, 50 mM Tris pH 7.5, 1 mM PMSF, 10 μg/ml leupeptin, and 100 μg/ml aprotinin). The extracts were placed on ice for 15 min and centrifuged to remove cellular debris. The protein amount of supernatants was determined using a Bradford assay (Bio-Rad Laboratories, Mississau- ga, ON, Canada). Thirty micrograms of total protein was run on 10% SDS-PAGE gels and electrophoresed into a nitrocellulose membrane (Amer- sham Pharmacia Biotech). The membrane was immunoblotted using a mouse monoclonal antibody specific to the phosphorylated p44/p42 MAPK (P-MAPK, Thr202/Tyr204) (New England Biolabs) [31]. Alternatively, the membrane was probed with a rabbit polyclonal antibody for p44/42 MAPK (New England Biolabs), which detects total MAPK (T-MAPK, phosphorylation state independent) levels. After washing, the signals were detected with horseradish peroxidase-conjugated secondary antibody and visualized using an enhanced chemiluminescent system (Amer- sham Pharmacia Biotech). P-MAPK levels were quantitated using a computerized visual light densitometer (model 620, BioRad Laboratories) and normalized against the levels of T-MAPK per sample. MAPK activity was represented as an P-MAPK-T:MAPK ratio.
Expression of P2U-R mRNA in pre-neoplastic and neoplastic OSE cells was investigated by RT-PCR. A predicted 599-bp PCR product of P2U-R was obtained and confirmed by sequence analysis (data not shown). Human granulosa-luteal cells were used as a positive control. Mw, Molecular weight; Tm(−), PCR reaction without template.

**FIG. 1.** Expression of P2U-R mRNA in pre-neoplastic and neoplastic OSE cells was investigated by RT-PCR. A predicted 599-bp PCR product of P2U-R was obtained and confirmed by sequence analysis (data not shown). Human granulosa-luteal cells were used as a positive control. Mw, Molecular weight; Tm(−), PCR reaction without template.

**FIG. 2.** Effect of ATP on proliferative index. To evaluate the role of ATP in pre-neoplastic and neoplastic OSE cells, the cells were treated with increasing concentrations (0.1, 1, 10, 100, and 1000 μM) of ATP for 24 h, and a [%H]thymidine incorporation assay was performed in IOSE-29, IOSE-29EC, and OVCAR-3 cells. Data are shown as the means of two individual experiments performed in triplicate (n = 2) and are presented as the mean ± SD. a, P < 0.05 vs. untreated control; b, P < 0.05 vs. 100 μM ATP treatment.

**FIG. 3.** Effect of ATP on MAPK activation. A time-dependent experiment was performed following treatment with 100 μM ATP on MAPK activity in IOSE cell lines. Data are shown as the means of three individual experiments and are presented as the mean ± SD. a, P < 0.05 vs. control (0 min).

**RESULTS**

**Expression of P2U-R mRNA**

The mRNA expression of P2U-R in preneoplastic and neoplastic OSE cells was investigated by RT-PCR. Human granulosa-luteal cells (hGLCs) were used as positive controls [15]. A predicted PCR product of P2U-R was obtained at 599 base pairs (bp) and confirmed by sequence analysis (data not shown). As demonstrated in Figure 1, the P2U-R mRNAs are expressed in IOSE cell lines (IOSE-29 and IOSE-29EC) and OVCAR-3.

**Effect of ATP on Proliferative Index**

Treatments with 1, 10, 100, and 1000 μM ATP resulted in significant growth stimulation in IOSE-29 cells, and 1, 10, and 100 μM ATP also induced growth stimulation in IOSE-29EC cells (Fig. 2). Growth stimulation was also observed following ATP treatment in OVCAR-3 cells (Fig. 2). It appears that a high dose of ATP (1000 μM) reversed growth stimulation in all cell lines, but it was significant only in IOSE-29 cells (Fig. 2).

**Effect of ATP on MAPK Activation**

A time-dependent experiment was performed following treatment with 100 μM ATP on MAPK activity in IOSE cell lines. As shown in Figure 3, A and B, treatment with ATP induced a significant, twofold to threefold increase in P-MAPK at 5–20 min in IOSE-29 cells. Activated MAPK declined to control levels after 30 min in these cells. Treatment with ATP resulted in a significant, similar MAPK activation after 5 min, and the maximum effect was observed at 20 min by increasing 3.7-fold MAPK activation in IOSE-29EC cells (Fig. 3B). MAPK activation by ATP was sustained for 60 min in IOSE-29EC cells.

**Data Analysis**

Data are shown as the means of three individual experiments and presented as means ± SD. Data were analyzed by one-way ANOVA followed by the Tukey multiple comparison test or the Dunnett test. Differences were considered statistically significant at *P* < 0.05.
FIG. 4. Effect of PD98059 and staurosporin on ATP-induced MAPK activation. To examine the role of ATP on MAPKs in IOSE cell lines, the cells were pretreated with 50 μM PD98059 or 0.1 μM staurosporin for 30 min, followed by treatment with 100 μM ATP for 10 min. Data are shown as the means of three individual experiments and are presented as the mean ± SD. a, P < 0.05 vs. untreated control; b, P < 0.05 vs. 100 μM ATP treatment; c, P < 0.05 vs. 50 μM PD98059 or 0.1 μM staurosporin treatment.

Effects of ATP and PD98059/Staurosporin on Elk-1 Phosphorylation

The Ets family transcription factor, Elk-1, is a physiological substrate for p42 MAPK and p44 MAPK [32, 33]. To investigate whether ATP-induced activation of MAPK leads to phosphorylation of Elk-1 in vitro, the cells were treated with 100 μM ATP for 10 min in the presence or absence of 50 μM PD98059 or 0.1 μM staurosporin for 30 min before ATP treatment. As shown in Figure 5, treatment with ATP resulted in a significant increase in Elk-1 phosphorylation, whereas pretreatment with PD98059 or staurosporin completely attenuated ATP-induced Elk-1 phosphorylation in both IOSE-29 and IOSE-29EC cells.

Effect of MAPK Inhibitor on ATP-Stimulated Cell Growth

To evaluate the effect of MAPK inhibitors on ATP-stimulated cell growth, the cells were pretreated with 50 μM PD98059 or 0.1 μM staurosporin for 30 min and then treated with 100 μM ATP for 24 h in IOSE cell lines and OVCAR-3 cells. An [3H]thymidine incorporation assay was performed as previously described in Materials and Methods. As expected, treatment with 100 μM ATP resulted in significant growth stimulation in these cells (Fig. 6). In addition, pretreatment with PD98059 or staurosporin completely reversed ATP-stimulated cell growth in IOSE-29, IOSE-29EC, and OVCAR-3 cells (Fig. 6).
DISCUSSION

Purinergic receptors have been classified as P1 and P2 receptors. P1 receptors have a high pharmacologic affinity for extracellular adenosine and AMP (adenosine > AMP > ADP > ATP), whereas P2 receptors have high affinity for ATP and ADP (ATP > ADP > AMP > adenosine) [4, 6, 7]. Six subtypes of P2 purinergic receptors (P2X, P2Y, P2D, P2T, P2Z, and P2U) have been identified in pharmacological and molecular cloning studies [34]. Considering that the ovary is a well-innervated organ, it is tempting to speculate that coreleased ATP from nerve endings may play a role in regulation of cellular proliferation and response in normal or neoplastic OSE cells if these cells express ATP receptors. The concentration of ATP in granules of sympathetic nerves and in acetylcholine-containing granules of parasympathetic nerves can be as high as 150 mM [35]. Exocytotic release of ATP has also been demonstrated in nonneuronal cells, including platelets [36], adrenal chromaffin cells [37], mast cells [38], and basophilic leukocytes [39]. Although ATP is present in millimolar concentrations in the cytosol, extracellular levels of the nucleotide will normally be maintained at very low levels by the ubiquitous ecto-ATP diphosphohydrolase and ecto-ATPase [39–41]. Further, micromolar levels of ATP have been demonstrated to exert physiologic effects on human granulosa-luteal cells [17].

Little information is available regarding the expression of ATP receptors and the precise role of ATP in preneoplastic and neoplastic OSE cells. It has been recently demonstrated that P2Y2 receptors were expressed in human ovarian carcinoma cell lines, EFO-21 and EFO-27, and that extracellular ATP resulted in a dose-dependent increase in intracellular calcium concentration in these cells [1]. Similarly, micromolar ranges of extracellular ATP resulted in calcium mobilization in human ovarian adenocarcinoma cell lines, OVCAR-3 and SKOV-3 [2, 3]. The present studies indicate that P2U-R was expressed in IOSE cell lines, which were directly derived from normal OSE cells transplanted with SV-40 large T antigen [13, 14], and OVCAR-3 cells, suggesting that ATP may play a role in these cells.

The effect of extracellular ATP on cell proliferation was examined using an [3H]thymidine incorporation assay in these studies. Treatments with 1, 10, 100, and 1000 M ATP resulted in significant growth stimulation in IOSE-29 cells, whereas three concentrations of ATP (1, 10, and 100 M) induced growth stimulation in IOSE-29EC cells. In addition, growth stimulation was observed following ATP treatments in OVCAR-3 cells. These results are in agreement with previous reports [2, 3] demonstrating that ATP inclusion in the medium significantly stimulated growth of OVCAR-3 and SKOV-3 cells. However, it has been demonstrated that a slowly degradable analogue, ATP-γ-S, attenuated basal and fetal calf serum-induced cell proliferation in a time- and dose-dependent fashion via its receptors in EFO-21 and EFO-27 cells, suggesting that the effect of ATP on cell proliferation is cell-specific in ovarian cancer cells. A high dose of ATP (1000 M) reversed growth stimulation compared with 100 M ATP treatment in IOSE-29EC cells, suggesting that the higher dose may have a biphasic effect or elicit a nonspecific response on cell proliferation in these cells.

Protein phosphorylation is a critical regulatory response to cellular proliferation and differentiation. The MAPK cascade is known to regulate acute cellular responses and to control transcriptional events through phosphorylation of target enzymes and transcriptional factors [19–21]. Activation of ERK is induced by phosphorylation of both threonine and tyrosine residues of the enzymes as a result of stimulation of Ras, ERK kinase kinase, MEK kinase, and MEK [19, 20, 42]. It has been demonstrated that MAPKs were regulated by cisplatin [25], paclitaxel [26], and endotheinin-1 [27] in ovarian cancer cells. In addition, treatment with GnRH analogue (GnRHa) resulted in an increase of ERK up to 24 h and suppression of ERK activation by PD98059, which binds MEK, blocked GnRHa-induced growth inhibition as well as hypophosphorylation of pRB in CaOv3 cells [28]. Considering that the mechanism of ATP action in ovarian tumors is unclear, we investigated the molecular mechanism of ATP-induced MAPK activation and its role in preneoplastic and neoplastic OSE cells. Involvement of the MAPK pathway by extracellular ATP has been suggested by recent studies, demonstrating that mitogenic effect of ATP or uridine triphosphate (UTP) was mediated through the ras/raf/MEK/MAPK pathway via a P2Y2 receptor in C6 glioma cells [43]. In addition, we previously reported that ATP activates MAPK subsequent to phospholipase C (PLC) and PKC activation through a pertussis toxin (PTX)-insensitive G-protein without affecting intracellular cAMP production in human granulosa-luteal cells [17]. In a time-dependent study, treatment with 100 M extracellular ATP resulted in MAPK activation at 5–10 min in IOSE-29 cells. Activated MAPK declined to control levels after 20 min in these cells. The same concentration of ATP induced MAPK activation after 5 min and sustained it for 60 min in IOSE-29EC cells. It appears that cellular responses to MAPK may be influenced by the duration of its activation. Sustained activation of MAPK is associated with cellular differentiation by nerve growth factor in PC12 cells, whereas transient activation of MAPK by epidermal growth factor (EGF) leads to cellular proliferation [44, 45]. Thus, a rapid activation of MAPK by ATP
in IOSE-29 and IOSE-29EC cells was related to growth stimulation in the present study. However, the cause of sustained response following ATP treatment in IOSE-29EC cells has yet to be elucidated. EGF stimulated an early rise in ERK activity at 4 min followed by a rapid decline in normal breast epithelium, whereas sustained ERK activity (1 h) was observed in neoplastic breast cells [46], suggesting that the time course of ERK activity may be different between normal and neoplastic cells. In addition, PD98059 inhibited EGF-stimulated proliferation and ERK activity in a parallel and dose-dependent manner, indicating that ERK activation is permissive for the proliferative response to EGF.

In the present study, extracellular ATP induced MAPK activation in both IOSE-29 and IOSE-29EC cells, whereas the effect of extracellular ATP was completely reversed by pretreatment with 50 μM PD98059, an MEK inhibitor, in both ATP-induced MAPK activation and ATP-stimulated proliferative index, suggesting that the growth stimulatory effect of ATP may be related to MAPK activation in pre-neoplastic and neoplastic OSE cells. In the previous reports, EGF activated ERK1/2, increased and sustained levels of c-jun mRNA, but it had no effect on JNK1 activation in IOSE-29 cells [47]. Similarly, EGF has been demonstrated to induce activation of ERK, and cellular proliferation was partially inhibited by PD98059 in a prostate cancer cell line [48]. In addition, it has been shown that EGF-induced cell proliferation, and matrix metalloproteinase (MMP)-9 induction and invasion through reconstituted basement membrane were significantly reduced when breast epithelial cells were exposed to the MEK inhibitor (PD98059) or MAPK inhibitors (apigenin or MAPK antisense phosphorothioate oligodeoxynucleotides). These results indicate that interference with MAPK activity may affect the growth and invasiveness of tumors in which the signaling cascade is activated [49]. In addition, staurosporin, a PKC inhibitor, was employed to investigate whether ATP-induced MAPK is mediated by a PKC-dependent pathway [50]. The activation of MAPK by ATP was completely blocked by pretreatment with staurosporin (0.1 μM) for 30 min in both cell lines, suggesting that a PKC pathway may play a role in ATP-induced MAPK activation in pre-neoplastic and neoplastic OSE cells. In agreement with the effect of PD98059 on ATP-induced cell proliferation, ATP-induced growth stimulation was significantly blocked by pretreatment with staurosporin in both IOSE-29 and IOSE-29EC cells. Thus, it is tempting to further investigate whether the PKC pathway may be involved in ATP-induced MAPK activation in pre-neoplastic and neoplastic OSE cells. In addition, treatment with PD98059 or staurosporin but without ATP treatment resulted in a decrease of MAPK basal levels compared to controls.

P2U-R belongs to a superfamly of G-protein-coupled receptors that interact with the intracellular signaling system through 7-transmembrane domains [51]. A transient increase of c-fos gene expression and MAPK activation were demonstrated in MCF-7 breast cancer cells in response to extracellular ATP [8] through activation of specific purinergic receptors. Several studies have shown that MAPKs phosphorylate ternary complex factor (TCF) proteins such as Elk-1 and SAP-1 [32, 33, 52]. The activated TCF protein regulates the expression of c-fos and other coregulated genes through their actions on the serum response element. Therefore, the ability of ATP to activate a downstream effector of the MAPK pathway was examined using the immunoprecipitation method. The present study demonstrated that treatment with ATP resulted in substantial phosphorylation of Elk-1 fusion protein in vitro. These results confirmed that ATP action is mediated by the activation of MAPK, because pretreatment with PD98059 or staurosporin completely reversed the effect of ATP on Elk-1 phosphorylation. These results taken together suggest that ATP-induced MAPK activation resulted in phosphorylation of Elk-1, the Ets family transcription factor, which possibly mediates cellular functions in response to ATP in pre-neoplastic and neoplastic OSE cells.

In conclusion, we demonstrated that P2U-R was expressed in IOSE and OVCAR-3 cells, and that extracellular ATP induced growth stimulation in these cells. In addition, treatment with ATP resulted in an activation of the MAPK cascade, presumably via the PKC pathway, and that it activated MAPK phosphorylated Elk-1 in IOSE-29 and IOSE-29EC cells. These results suggest that the MAPK cascade may be involved in cellular functions such as growth stimulation in response to ATP in pre-neoplastic and neoplastic OSE cells.

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