Crosstalk between adenosine receptor (A_{2A} isoform) and ERα mediates ethanol action in MCF-7 breast cancer cells

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Abstract. Alcohol consumption increases the risk of breast cancer but the underlying mechanisms are not well understood. We have shown previously that ethanol activates ER signalling pathway in a cAMP/PKA-mediated ligand-independent manner. Since the activation of A_{2A} adenosine receptor (A_{2A}AR) by ethanol has been reported in other cell types, here we tested if cross-talk between this Gs-coupled receptor and ERα could be involved in ethanol effects in breast cancer cells. Our study shows that A_{2A}AR is expressed and functional in the hormone-dependent breast cancer cell line MCF-7. Interestingly, activation of this receptor by the selective agonist CGS21680 stimulates the transcription of progesterone receptor, a well known estrogen target gene. CGS21680 also stimulates the pEREtkLuc reporter activity in transfected MCF-7 cells, an effect antagonized by the anti-estrogen ICI182,780. Moreover, CGS21680 stimulates the proliferation of MCF-7 cells similarly to E_2. Finally, the A_{2A}AR antagonist MSX-3 inhibits the ethanol-induced activation of ERα signalling pathway. These results demonstrate cross-talk between A_{2A}AR and ERα that is involved in ethanol action. This could open new perspectives for the therapy of estrogen-dependent breast cancer.

Introduction

Adenosine is a natural metabolite that plays a role in vasodilatation, cardioprotection after ischemia, inhibition of platelet aggregation, mast cell activation, and cell growth (1). Four adenosine receptors (ARs) (A_1AR, A_{2A}AR, A_{2B}AR and A_3AR) have been cloned (2). Each of them regulate the activity of adenylyl cyclase: the A_1 and A_3 receptors mediate a decrease in cAMP via Gi/o whereas the two A_2 receptors mediate an increase in cAMP via Gs (3). Extracellular adenosine activates A_1 and A_{2A} receptors whereas at high concentrations, it acts mainly on A_3AR.

Adenosine and AR seem to play different roles in various types of cancer (4). In breast cancer, selective A_1AR and selective A_3AR agonists inhibit cell proliferation of both hormone-dependent and hormone-independent breast cancer cell lines (5-8). An inhibitor of the ecto-5'-nucleotidase CD73, an enzyme that generates adenosine, decreases proliferation of breast cancer cells MDA-MB231 *in vitro* as well as *in vivo* in xenografts (9), while ecto-5'-nucleotidase overexpression promotes invasion, migration and adhesion of breast cancer cells (10). Breast carcinoma tissues show higher A_1 and A_3AR expression in the tumor vs. adjacent non-neoplastic tissue or normal tissue (11,12). Moreover, a depletion of A_1AR in MDA-MB-468 human breast tumor cells significantly induces apoptosis (12).

Breast cancer is the most common cancer in women. About 60% of all patients have hormone-dependent breast cancer. These tumors express estrogen receptors (ER) and require estrogens for their growth (13). ERα, a member of the superfamily of steroid nuclear receptors, mediates the effects of these hormones on tumor development. In the best understood mode of action, the liganded ERα receptor binds to specific estrogen response elements (EREs) within target genes and recruits a p160/p300 coactivator complex to the promoter (14,15). This coactivator complex promotes gene transcription by remodeling chromatin and by contacting the basal transcription machinery. A hormone-independent activation of ERα also occurs in human breast cancer cells. Indeed, ERα activity can be stimulated by several kinase pathways (16). For instance, the mitogen-activated protein kinase (MAPK) pathway activated by epidermal growth factor directly phosphorylates ERα in the absence of ligand and induces ER transactivation (17,18). In MCF-7 breast cancer cells, ethanol induces a ligand-independent activation of ERα mediated by the cAMP/PKA pathway (19). However, the mechanisms involved in the ethanol-induced activation of cAMP/PKA are unknown. Interestingly, in NG108-15 cells (neuroblastoma x glioma hybrid cells), acute ethanol exposure induces an increase in extracellular adenosine that activates A_2 receptors to stimulate cAMP production (20). Such a mechanism could exist in MCF-7 cells.

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In the present study, we show that the A_{2A} isoform of adenosine receptor is expressed by MCF-7 breast cancer cells. We demonstrate that activation of this receptor leads to the activation of ERα and to cell growth stimulation. A_{2A}AR also mediates the ethanol-induced activation of ERα signalling pathway.

Materials and methods

Materials. Dulbecco's modified Eagle's medium (DMEM), L-glutamine, 17-ß-estradiol (E_2), Forskolin, MSX-3 and CGS21680 were purchased from Sigma-Aldrich (Saint-Quentin Fallavier, France) and fœtal calf serum (FCS) from Eurobio (Les Ulis, France). ICI 182,780 was purchased from Tocris Cookson (Bristol, UK). H89 and SQ22536 were purchased from Calbiochem (La Jolla, CA). These chemicals were dissolved in ethanol.

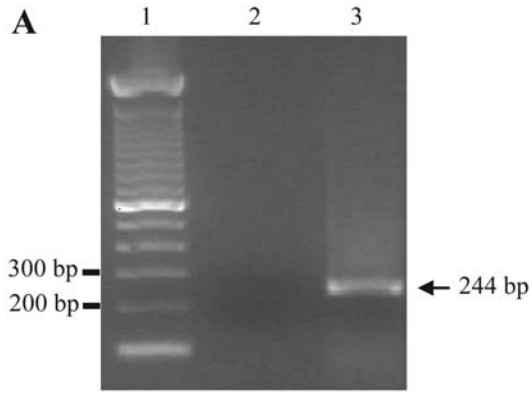
Cell culture treatment, proliferation assay and immunocytochemistry. MCF-7 cells were routinely grown in DMEM supplemented with 10% FCS and 2 mM L-glutamine, at 37˚C in a 5% (v/v) CO_2 humidified atmosphere.

Treatments were conducted as previously described (19). When ethanol was used as a control, its concentration was 0.1%. When we studied ethanol as a test compound, it was used at 0.3% For cell proliferation assay, after 48 and 96 h, cells were counted with the CellTiter-Glo™ Luminescent Cell Viability Assay (Promega, Charbonnieres, France). Each treatment was performed in triplicate.

For immunocytochemistry, MCF-7 cells were seeded on Poly L-lysine coated coverslips. Twenty-four hours after attachment, coverslips were washed with PBS and fixed in 4% PAF for 20 min. After washing in Tris-buffered saline, preparations were treated for A_{2A}AR detection using the 7F6-G5-A2 monoclonal antibody (Santa Cruz Biotechnology) (1:300) and an Alexa Fluor 555 conjugated-goat anti-mouse antibody (Invitrogen) (1:1000). Cells were counterstained with Hoechst dye to visualise nuclei. Preparations were analysed under U.V. illumination on an Eclipse 80i microscope (Nikon, Champigny sur Marne, France). Images were collected using LuciaG software 4.81 (Laboratory imaging).

Semi-quantitative RT-PCR. For progesterone receptor (PR) and ß-actin, the protocol for semi-quantitative RT-PCR has been described previously (19,21). The specific primers for A_{2A}AR were 5'-AACCTGCAGAACGTCACCAA-3' and 5'-GTCACCAAGCCATTGTACCG-3' (22). Its amplification was carried out with 30 cycles of amplification (94˚C for 40 sec, 65˚C for 1 min and 68˚C for 1 min). For each gene, the number of cycles was chosen to realize the analysis in the linear phase of the PCR reaction. The PCR products were quantified as previously described (19).

Transient transfection assays. MCF-7 cells were transfected with the expression plasmids using Exgen 500 as recommended by the manufacturer (Euromedex, France). The protocol for transfection experiments has been described previously (19,21). After transfection, cells were allowed to grow for one day and treated for 24 h in fresh medium.



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PCRp   1  GAACGTCACCAACTACTTTGTGGTGTTGCTCGCCATCCCC
          ||||||||||||||||||||||||||||||||||||||||
A2AAR 114 GAACGTCACCAACTACTTTGTGGTGTTGCTCGCCATCCCC

PCRp  41  TTTGCCATCACCATCAGCACCGGGTTCTGCGCTGCCTGCC
          ||||||||||||||||||||||||||||||||||||||||
A2AAR 154 TTTGCCATCACCATCAGCACCGGGTTCTGCGCTGCCTGCC

PCRp  81  ACGGCTGCCTCTTCATTGCCTGCTTCGTCCTGGTCCTCAC
          ||||||||||||||||||||||||||||||||||||||||
A2AAR 194 ACGGCTGCCTCTTCATTGCCTGCTTCGTCCTGGTCCTCAC

PCRp  121 GCAGAGCTCCATCTTCAGTCTCCTGGCCATCGCCATTGAC
          ||||||||||||||||||||||||||||||||||||||||
A2AAR 234 GCAGAGCTCCATCTTCAGTCTCCTGGCCATCGCCATTGAC

PCRp  161 CGCTACA 167
          |||||||
A2AAR 274 CGCTACA 280
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Figure 1. A_{2A}AR mRNA expression in MCF-7 cells. (A) Agarose gel electrophoresis showing the 244-bp product obtained after RT-PCR with A_{2A}AR specific primers. Lane 1, DNA ladder; lane 2, negative control; lane 3, MCF-7 cDNA. (B) Alignment showing 100% identity between the sequence of the PCR product (PCRp) and that of human A_{2A}AR (NM_000675.4).

SEAP (secreted alkaline phosphatase), luciferase and ß-Galactosidase (ß-Gal) activities were measured as previously described (19,21).

Statistical analysis. The results are expressed as mean ± standard error of several experiments as indicated in the text. Differences among groups were tested using analysis of variance (ANOVA). Differences at $P<0.05$ were considered statistically significant. The pecific post-hoc comparisons between treatment groups were examined with the Student Newman's Keuls test (SPSS v11.0 Computer Software).

Results

A functional A_{2A}AR is expressed in MCF-7 cells. RT-PCR analyses showed the presence of the A_{2A}AR mRNA in MCF-7 cells (Fig. 1). The amplified product was of the expected size of 244 bp and its identity was confirmed by sequencing (Fig. 1A and B). The presence of the protein was demonstrated by the use of an anti-A_{2A}AR antibody that stained the membrane of MCF-7 cells in immunocytochemistry experiments (Fig. 2A). Moreover, in MCF-7 cells transfected with the reporter construct pCRE-SEAP (with 3 tandem copies of the cAMP Response Element (CRE) consensus sequence), the treatment with the A_{2A}AR selective

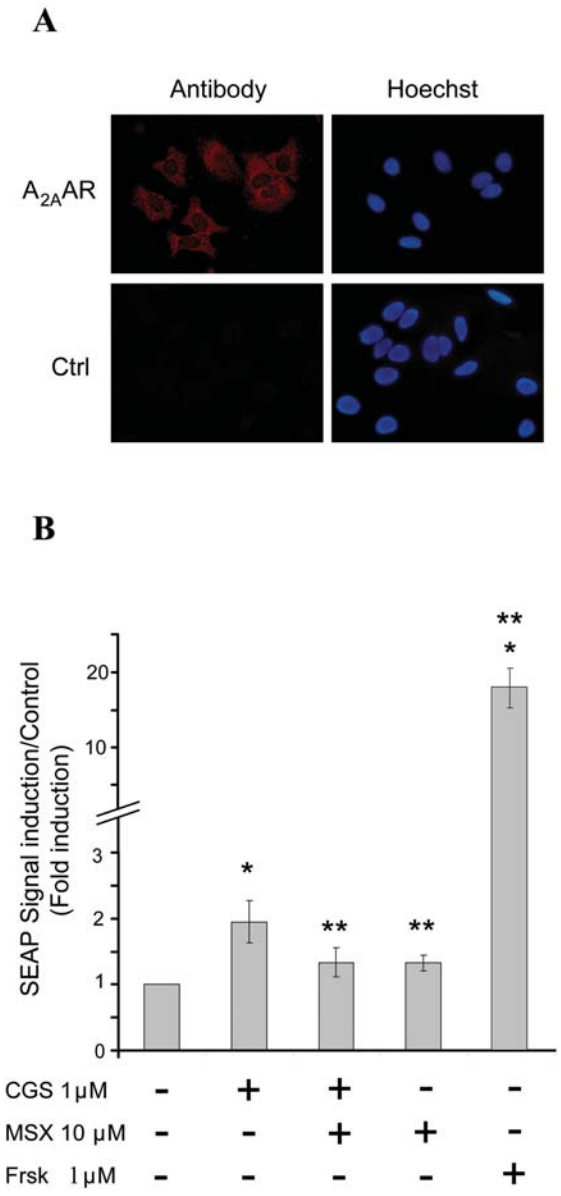

Figure 2. $A_{2A}AR$ protein expression in MCF-7 cells. (A) Immunodetection of $A_{2A}AR$ in MCF-7 cells leading to membrane staining. In control cells the primary antibody was omitted. (B) Expression of the SEAP reporter gene following treatment of pCRE-SEAP and pCMV-ßGal co-transfected MCF-7 cells with the $A_{2A}AR$ agonist CGS21680 for 24 h in presence or absence of the $A_{2A}AR$ antagonist MSX-3. Forskolin (Frsk) treatment was used as a positive control. SEAP reporter activity was normalized with the ß-Gal activity. *Significantly different from untreated control cells ($P<0.05$). **Significantly different from CGS-treated cells ($P<0.05$).

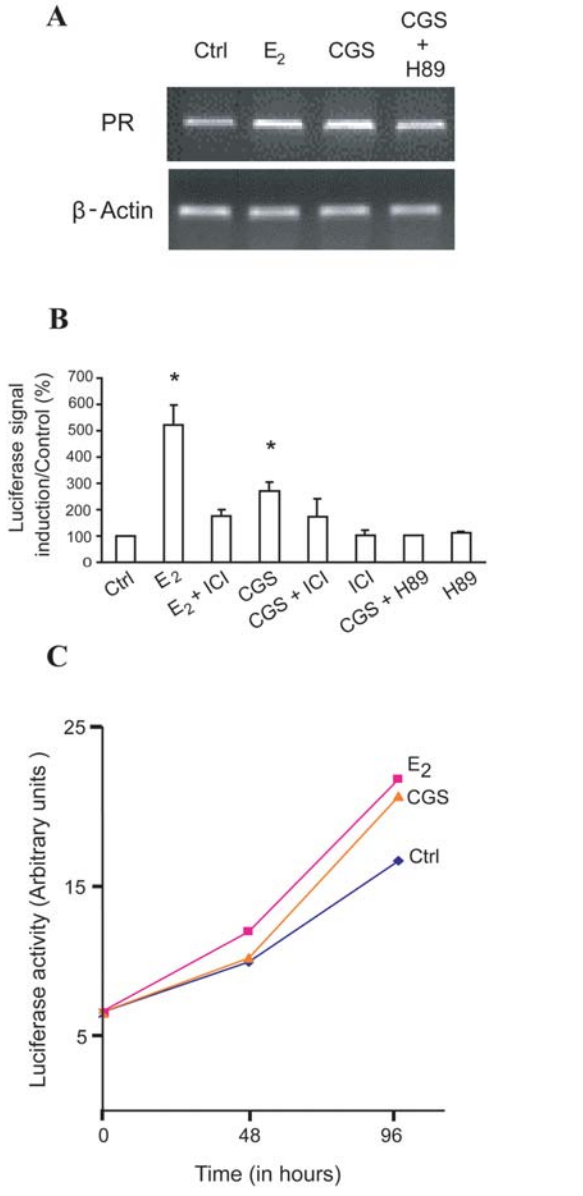

Figure 3. $A_{2A}AR$ activation stimulates ERα target gene expression. (A) Semi-quantitative RT-PCR analysis of PR mRNA. MCF-7 cells were treated for 24 h with either E_2 (1 nM) or the selective $A_{2A}AR$ agonist CGS21680 (1 μM) in presence or absence of the PKA inhibitor H89 (10 μM). ß-actin mRNA levels were determined as an internal control. (B) Expression of the luciferase reporter gene following 24 h treatment of pERE-tkLuc and pCMV-ßGal co-transfected MCF-7 cells with the $A_{2A}AR$ agonist CGS21680 or E_2, in presence or absence of ICI182,780 or H89. Luciferase reporter activity was normalized with the ß-Gal activity and expressed as percentage of the control assumed as 100%. *Significantly different from untreated control cells ($P<0.05$). (C) Cell proliferation analysis. MCF-7 cells were treated with CGS21680 ($A_{2A}AR$ agonist, 1 μM), E_2 (1 nM), or ethanol (control, 0.1%). Results are expressed in arbitrary units. The mean of 3 different experiments is shown without standard error of the mean for a better observation of the different points.

agonist CGS21680 induced 1.95-fold increase in SEAP expression (Fig. 2B). This effect was inhibited by the antagonist MSX-3 (Fig. 2B).

$A_{2A}AR$ activation stimulates ERα signalling pathway. To test the hypothesis of a cross-talk between $A_{2A}AR$ and ERα, we studied if the expression of the estrogen target gene PR (19,21) could be stimulated by an $A_{2A}AR$ agonist. We observed an increased level of PR mRNA following a 24 h treatment with 1 μM CGS21680. This effect was abolished in the presence of the PKA inhibitor H89 (Fig. 3A). In order to definitely demonstrate the activation of ERα target gene expression by the $A_{2A}AR$ agonist, MCF-7 cells were transfected with the reporter construct pEREtkLuc and treated by different compounds (Fig. 3B). Similarly to E_2 (1 nM) used as a control, the $A_{2A}AR$ agonist CGS21680 (1 μM) significantly

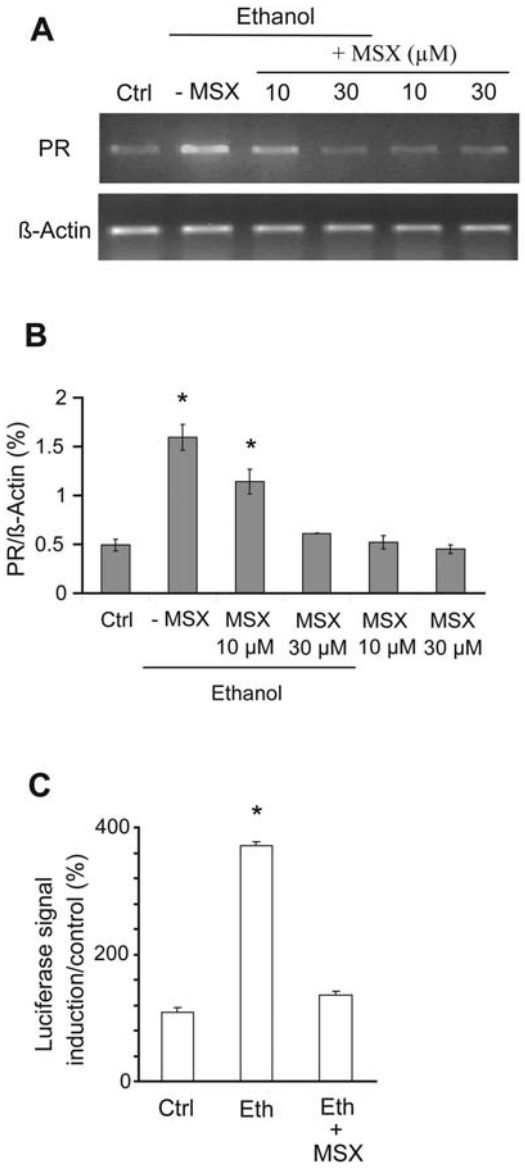

Figure 4. A_{2A}AR mediates ethanol-induced activation of ER signalling pathway. (A) Semi-quantitative RT-PCR analysis of PR mRNA. MCF-7 cells were treated for 24 h with ethanol (0.3%) and a selective A_{2A}AR antagonist, MSX-3 (MSX) (10 and 30 μM). ß-actin mRNA levels were determined as an internal control. (B) The histogram represents the mean ± SE of four separate RT-PCR experiments in which the band intensities of PR were evaluated in terms of optical density arbitrary units and normalized with the ß-actin signal intensity. *Significantly different from untreated control ($P<0.05$). (C) MCF-7 cells were cotransfected with pEREtkLuc and pCMV-ßGal. The cells were treated for 24 h with ethanol (0.3%) in the presence or absence of MSX-3. Luciferase reporter activity was normalized with the ß-Gal activity and expressed as percentage of the control assumed as 100%. In each experiment, the activities of transfected plasmids were assayed in triplicate transfections. *Significantly different from untreated control ($P<0.05$).

increased luciferase activity (3-fold). This effect was mediated by the activation of ERα since it was antagonized by the pure antiestrogen ICI182,780 (100 nM) which also inhibited the response to E_2. Besides, the stimulation of ERE-directed expression by CGS21680 was inhibited in presence of the PKA inhibitor H89 (Fig. 3B).

We also determined if the cross-talk between A_{2A}AR and ERα could stimulate cell proliferation. After 96 h, CGS21680 (1 μM) stimulated MCF-7 cell proliferation (Fig. 3C). A similar effect was observed with E_2 (1 nM) (Fig. 3C). Taken together, these results demonstrate cross-talk between A_{2A}AR and ERα.

A_{2A}AR mediates ethanol-induced activation of ERα signalling pathway. We have recently shown in MCF-7 cells that ethanol activates the cAMP/PKA pathway and induces a ligand-independent activation of ERα (19). Interestingly, ethanol has been reported to stimulate cAMP production in both NG 108-15 and lymphoma cells via the A_{2A}AR (20). In order to test the involvement of A_{2A}AR in ethanol-induced activation of ERα signalling pathway in MCF-7 cells, PR gene expression was studied after treatment with ethanol for 24 h, in presence or absence of MSX-3 (10 and 30 μM). Ethanol was used at 0.3% since it is the most efficient dose *in vitro* (19). The antagonist induced a dose-dependent inhibition of ethanol-induced increase in PR expression (Fig. 4A and B).

We also studied pEREtkLuc transfected MCF-7 cells (Fig. 4C). This transfection assay showed an inhibition of ethanol action in the presence of the A_{2A}AR antagonist MSX-3 (30 μM).

Discussion

Although the role played by adenosine and its receptors in breast cancer is not well understood, several studies indicate that adenosine is able to promote tumor cell proliferation and migration (10,23). Nevertheless, the underlying mechanisms remain obscure. In this study, we tested the hypothesis of cross-talk between A_{2A}Anenosine receptor and ERα in the homone-dependent breast cancer cell line MCF-7. Such cross-talk could explain some tumorigenic processes involving adenosine or compounds as ethanol which could increase adenosine levels (20).

Our results based on both mRNA and protein detection show that A_{2A}AR is expressed in MCF-7 cells. Besides, in the transfection assays using a pCRE-SEAP reporter construct, the data obtained following treatment of the cells with a selective A_{2A}AR agonist are in agreement with the increase in cAMP usually observed following A_{2A}AR activation (3). Hence, the A_{2A}AR pathway is functional in MCF-7 cells. These findings contrast with a previous study concluding that MCF-7 cells did not contain adenosine receptors as judged by the lack of effect of non-selective agonists on adenylyl cyclase activity or intracellular Ca^{2+} levels (24). However, the same author reported recently the presence of the transcript of all adenosine receptors in the same cell line, with A_3 and A_{2B} isoforms being functional as deduced from cAMP production (25).

Then, different approaches led to the demonstration of cross-talk between A_{2A}AR and ERα. First, the treatment of MCF-7 cells with an A_{2A}AR agonist induced an increase in PR mRNA level that is often studied as an ER target gene (19,21). This result was confirmed by a more precise test using transfection assays with a pEREtkLuc reporter construct. In this study it was surprising to observe that the stimulation of luciferase activity was in the same range in case of exposure to either an A_{2A}AR agonist or E_2 itself. This similar intensity was correlated with the results of proliferation analyses.

Finally, we observed that the A_{2A}AR antagonist MSX3 inhibited both the increase in PR expression and the ERE-mediated increase in luciferase activity in transfection assays that are usually observed following exposure of MCF-7 cells to ethanol. This demonstrated the important role of A_{2A}AR in the ethanol-induced activation of ERα signalling pathway (19). So, ethanol acts in a similar A_{2A}AR-dependent manner in breast cancer cells and in NG 108-15 and lymphoma cells (20).

To conclude, our results demonstrate cross-talk between A_{2A}AR and ERα that is involved in ethanol action on MCF-7 cells. Nevertheless, the link between ethanol and A_{2A}AR remains to be determined. In S49 lymphoma cells, ethanol exposure increases extracellular adenosine by inhibiting the adenosine uptake via the nucleoside transporter (20). A similar process could be involved in ethanol-treated MCF-7 cells to explain A_{2A}AR activation. Besides, A_{2A}AR is linked to estrogen signalling pathway, it can be activated by extracellular adenosine whose concentration is elevated in breast tumor environment (23), therefore A_{2A}AR could constitute an interesting target for the design of new therapeutic agents.

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References

1. Linden J: Molecular approach to adenosine receptors: receptor-mediated mechanisms of tissue protection. Annu Rev Pharmacol Toxicol 41: 775-787, 2001.
2. Fredholm BB, Ijzerman AP, Jacobson KA, Klotz KN and Linden J: International Union of Pharmacology. XXV. Nomenclature and classification of adenosine receptors. Pharmacol Rev 53: 527-552, 2001.
3. Schulte G and Fredholm BB: Signalling from adenosine receptors to mitogen-activated protein kinases. Cell Signal 15: 813-827, 2003.
4. Merighi S, Mirandola P, Varani K, Gessi S, Leung E, Baraldi PG, Tabrizi MA and Borea PA: A glance at adenosine receptors: novel target for antitumor therapy. Pharmacol Ther 100: 31-48, 2003.
5. Colquhoun A and Newsholme EA: Inhibition of human tumour cell proliferation by analogues of adenosine. Cell Biochem Funct 15: 135-139, 1997.
6. Lu J, Pierron A and Ravid K: An adenosine analogue, IB-MECA, down-regulates estrogen receptor alpha and suppresses human breast cancer cell proliferation. Cancer Res 63: 6413-6423, 2003.
7. Panjehpour M and Karami-Tehrani F: An adenosine analog (IB-MECA) inhibits anchorage-dependent cell growth of various human breast cancer cell lines. Int J Biochem Cell Biol 36: 1502-1509, 2004.
8. Chung H, Jung JY, Cho SD, Hong KA, Kim HJ, Shin DH, Kim H, Kim HO, Shin DH, Lee HW, Jeong LS and Kong G: The antitumor effect of LJ-529, a novel agonist to A3 adenosine receptor, in both estrogen receptor-positive and estrogen receptor-negative human breast cancers. Mol Cancer Ther 5: 685-692, 2006.
9. Zhou X, Zhi X, Zhou P, Chen S, Zhao F, Shao Z, Ou Z and Yin L: Effects of ecto-5'-nucleotidase on human breast cancer cell growth *in vitro* and *in vivo*. Oncol Rep 17: 1341-1346, 2007.
10. Wang L, Zhou X, Zhou T, Ma D, Chen S, Zhi X, Yin L, Shao Z, Ou Z and Zhou P: Ecto-5'-nucleotidase promotes invasion, migration and adhesion of human breast cancer cells Cancer Res Clin Oncol 134: 365-372, 2008.
11. Madi L, Ochaion A, Rath-Wolfson L, Bar-Yehuda S, Erlanger A, Ohana G, Harish A, Merimski O, Barer F and Fishman P: The A3 adenosine receptor is highly expressed in tumor versus normal cells: potential target for tumor growth inhibition. Clin Cancer Res 10: 4472-4479, 2004.
12. Mirza A, Basso A, Black S, Malkowski M, Kwee L, Pachter JA, Lachowicz JE, Wang Y and Liu S: RNA interference targeting of A1 receptor-overexpressing breast carcinoma cells leads to diminished rates of cell proliferation and induction of apoptosis. Cancer Biol Ther 4: 1355-1360, 2005.
13. Lykkesfeldt AE and Briand P: Indirect mechanism of oestradiol stimulation of cell proliferation of human breast cancer cell lines. Br J Cancer 53: 29-35, 1986.
14. Hall JM and McDonnell DP: Coregulators in nuclear estrogen receptor action: from concept to therapeutic targeting. Mol Interv 5: 343-357, 2005.
15. Singh RR and Kumar R: Steroid hormone receptor signaling in tumorigenesis. J Cell Biochem 96: 490-505, 2005.
16. Coleman KM and Smith CL: Intracellular signaling pathways: nongenomic actions of estrogens and ligand-independent activation of estrogen receptors. Front Biosci 6: 1379-1391, 2001.
17. Kato S, Endoh H, Masuhiro Y, Kitamoto T, Uchiyama S, Sasaki H, Masushige S, Gotoh Y, Nishida E, Kawashima H, Metzger D and Chambon P: Activation of the estrogen receptor through phosphorylation by mitogen-activated protein kinase. Science 270: 1491-1494, 1995.
18. Bunone G, Briand PA, Miksicek RJ and Picard D: Activation of the unliganded estrogen receptor by EGF involves the MAP kinase pathway and direct phosphorylation. EMBO J 15: 2174-2183, 1996.
19. Etique N, Flament S, Lecomte J and Grillier-Vuissoz I: Ethanol-induced ligand-independent activation of ERα mediated by cyclic AMP/PKA pathway: an *in vitro* study on MCF-7 breast cancer cells. Int J Oncol 31: 1509-1518, 2007.
20. Nagy LE, Diamond I, Casso DJ, Franklin C and Gordon AS: Ethanol increases extracellular adenosine by inhibiting adenosine uptake via the nucleoside transporter. J Biol Chem 265: 1946-1951, 1990.
21. Lecomte J, Flament S, Salamone S, Boisbrun M, Mazerbourg S, Chapleur Y and Grillier-Vuissoz I: Disruption of ERα signalling pathway by PPARγ agonists: evidences of PPARγ-independent events in two hormone-dependent breast cancer cell lines. Breast Cancer Res Treat 112: 437-451, 2008.
22. Christofi FL, Zhang H, Yu JG, Guzman J, Xue J and Kim M: Differential gene expression of adenosine A1, A2a, A2b, and A3 receptors in the human enteric nervous system. J Comp Neurol 439: 46-64, 2001.
23. Mujoomdar M, Bennett A, Hoskin D and Blay J: Adenosine stimulation of proliferation of breast carcinoma cell lines: evaluation of the [^{3}H]thymidine assay system and modulatory effects of the cellular microenvironment in vitro. J Cell Physiol 201: 429-438, 2004.
24. Panjehpour M, Castro M and Klotz KN: Human breast cancer cell line MDA-MB-231 expresses endogenous A2B adenosine receptors mediating a Ca^{2+} signal. Br J Pharmacol 145: 211-218, 2005.
25. Panjehpour M and Karami-Tehrani F: Adenosine modulates cell growth in the human breast cancer cells via adenosine receptors. Oncol Res 16: 575-585, 2007.