Adenosine Receptor Expression in Two Different Human Cancer Cell Lines at Molecular Level

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Abstract

Background: The metabolically active tumor cells may be characterized by a pronounced adenosine release that regulates the growth and development of the tumor. Consequently, the expression pattern of defined receptor subtypes will be an important determinant for specific effects of adenosine on the control of tumor cell growth. In recent studies, the expression profile, signal transduction, molecular function and cell growth modulation of adenosine receptors in the human breast cancer cell lines has been reported. To investigate the possible roles of adenosine receptors in other types of human cancers, in this study, we characterized the expression profile of adenosine receptors in two different human cancer cell lines: prostate carcinoma cell line (Du-145) and lung adenocarcinoma cell line (Calu-6). Our purpose is to test the hypothesis that diverse human cancer cell lines, according to their adenosine receptor subclass status, would show differential growth modulation.

Methods: RNA was extracted and reverse transcribed to cDNA. PCR primers were synthesized from human adenosine receptor cDNA sequences. PCR was performed under optimized condition for each receptor subtype. The PCR products were separated on agarose gels.

Results: All two human cancer cell lines studied contained detectable amounts of mRNA specific for adenosine receptor except A_3 subtypes.

Conclusion: In conclusion the differentially expressed genes identified in this study might provide new insights into the possible roles of adenosine receptors on cell growth and development.

Key Words: Prostate; lung; Human cancer; Cell line; RT-PCR; Adenosine receptor

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Introduction

Adenosine is known to regulate many cellular functions including cell proliferation and death. The cytotoxic effects of adenosine as well as its analogs (mainly through adenosine receptors) on a number of tumor and normal cells have attracted significance interest [1,2]. Among many diverse and well known of physiological, neurological, biochemical and pharmacological effects of adenosine receptors, Recently there has been a large attention given to elucidate the expression profile and functional role of 4 subgroups of adenosine receptors (namely A_1, A_{2A}, A_{2B} and A_3) in the modulation of cell proliferation and apoptosis of many different cell types as well as the formation, growth and development of tumor cells [3-9]. Consequently, the presence of defined receptor subtypes will be an important determinant for a specific effect of adenosine on the function of a tumor cell.

Although prostate cancer is the most prevalent cancer and the second leading cause of cancer death in men ,the molecular mechanism underlying the initiation, promotion and progression of prostate cancer remains largely unknown[10].

Lung cancer is the second most diagnosed cancer in men and women (after prostate and breast, respectively), and is the number one cause of death from cancer each year in both sexes. Although cigarette smoking and other minor factors are the main cause of most lung cancers, there are often low attention to explore the role of other factors in the development, diagnosis and treatment of lung cancer [11].To our knowledge, no studies evaluating the expression of adenosine receptors in the human prostate (DU-145) and human lung (Calu-6) cancer

cell lines have been carried out. Thus, the aim of this study was to evaluate the expression profile of adenosine receptors in these two cell lines.

Materials and Methods

Materials

Tissue culture media and Fetal Calf Serum were obtained from Gibco, flasks and plates from Nunc. Materials used for RT-PCR from Fermentas.

Cell lines and cell culture conditions

Human prostate carcinoma cell line (Du-145) and human lung adenocarcinoma cell line (Calu-6) were obtained from Pasture Institute at Tehran. The cell lines were grown in RPMI-1640 media supplemented with10% Fetal Calf Serum, 100 U/ml penicillin and 100µg/ml streptomycin in a humidified 5% CO2 atmosphere at 37°C. Cells were passaged two or three times weekly at a ratio between 1:4.

Primer Designing

All oligonucleotides used as primers in polymerase chain reaction (PCR) were used based on the published human adenosine receptors sequence specific for each target cDNA (Ref-Seq). Primer sequences were designed using Primer3 (http://biocore.unl.edu/cgi-bin/primer3) and Primer Bank (http://pga.mgh.harvard.edu/primerbank/) softwares. PCR primers were synthesized by Fazapaj ouh and are listed in table 1.

RNA preparation and Isolation

Exponentially growing cell monolayers were washed twice with PBS, and then harvested with a solution of trypsin-EDTA. After centrifugation for 3 min at 1,500 rpm, total cellular RNAs were extracted from 1-2 x 10^6 cell pellets by the acid guanidinium thiocyanate–phenol–chloroform method.

RT-PCR analysis for detection of adenosine receptor mRNA transcripts

Total RNA (5 µl) was reverse transcribed into cDNA using the M-MulV reverse transcriptase (1 µl), random hexamer (5 µl) as well as RNAse inhibitor and dNTPs (according to the standard protocol) at 42°C for 60 min. Single strand cDNA products were denatured and subjected directly to PCR amplification. For PCR, the targeted sequence was amplified in a 25 µl reaction volume containing: 3 µl target cDNA, 1 µl of each dNTP, 0.5 µl of each oligonucleotide primer, 1X PCR buffer and 1 U of Taq polymerase. Reaction mixtures were overlaid with mineral oil and PCR amplification was started by placing the capped tubes on the block in the DNA thermal cycler and adjusted to: 1 precycle for 2 min at 94° C, 35 cycles for 30 s at 94°C (denaturation), 45s at 56°C(annealing), 75s at 72°C(polymerization) and 1 cycle for 5 min at 72° C. Five microliters of each PCR reaction were subjected to electrophoresis on a 1.5% agarose gel and were visualized by ethidium bromide staining.

As negative controls, distilled water instead of cDNA or RT reactions without reverse transcriptase was subjected to PCR and we confirmed no false positive reaction. Quality of RNA and efficiency of the reverse transcriptase step were assessed by concurrent application of beta-actin transcripts from the same cDNA. Each experiment repeated three times with the same results.

Results

Identification of Adenosine receptors mRNA by RT-PCR analysis in the human lung adenocarcinoma cell line (Calu-6)

We used primers to specifically amplify the fragments of the human adenosine receptor cDNAs. As shown in Figure 1A, RT-PCR analysis of total RNA prepared from the cells revealed the expression of the adenosine A_1, A_{2A} and A_{2B} receptors but no expression for A_3 subtypes in the human lung adenocarcinoma cell line (Calu-6). The amplified products were of the appropriate size. Nevertheless, different intensities could be observed between the all receptor subtypes. Each experiment was reproduced several times with similar results. After reverse transcriptase was omitted in the RT reaction, no amplification products were observed. Moreover the amplification of beta actin mRNA was used as control. As shown in Figure 1B, the lack of expression of A_3 mRNA in the obtained results was confirmed by using another set of specific primers for A_3 adenosine receptor gene.

Identification of Adenosine receptors mRNA by RT-PCR analysis in the human prostate carcinoma cell line (Du-145)

Detection of adenosine receptor subtypes mRNA by reverse transcriptase PCR was performed in the human prostate carcinoma cell line (Du-145). The 300 and 237 bp amplified sequence corresponding to the A_1 and A_{2A}, respectively, adenosine receptors mRNA reverse transcribed into cDNA was reproducibly detected. Similar to the human lung adenocarcinoma cell line (Calu-6), no expression was observed for A_3 subtype in this cell line (Fig 2A). As shown in Figure 2A, we saw a multiple PCR products for A_{2B} receptor. However, changing the PCR condition wasn't changed the results. By using another

Table 1. Sequences of the primer pairs for human adenosine receptors and human housekeeping genes used for RT-PCR

Target Name	Accession No. (Ref-Seq)	Primer Sequence Forward Primer Sequence Reverse	RT-PCR Product (bp)
A_1	NM-000674	5´-tccctctccggtacaagatg-3´ 5´-gctgcttgcggattaggtag-3´	300
A_{2A}	NM-000675	5'-agctgaagcagatggagagc-3' 5'- agggattcacaaccgaattg-3'	237
A_{2B}	NM-000676	5'-cagcgggagatccatgcag-3' 5'-cggttccggtaagcatagacaat-3' 5'-GCTCCATCTTCAGCCTTCTG-3' 5'-CATGCACAGGTAACCAGCAC-3'	206 483
A_3	NM-000677	5'-tacccacgcctccatcatgt-3' 5'- ggggtcaatcccaccagga -3' 5'-GGGCATCACAATCCACTTCT-3' 5'-AGGGCCAGCCATATTCTTCT-3'	156 171
Beta- Actin	NM-001101	5'- agaaaatctggcaccacacc -3' 5'- aggaaggaaggctggaagag -3'	553

set of primers for A_{2B}, a single and sharp band was obtained (Fig2B).

Discussion

The knowledge of the expression pattern of different tumor cells is essential for the development of potential therapeutic targets that may aid in more efficient tumor growth control. Recently we have identified the expression profile, signal transduction; molecular function and cell growth modulation of adenosine receptor subtypes in the human breast cancer cell lines [4-5].No study identified or characterized adenosine receptors at the molecular level in the human lung and prostate cancers. To investigate the possible roles of adenosine receptors in other types of human cancers, in this study, we characterized the expression profile of adenosine receptors in the human lung adenocarcinoma cell line (Calu-6) and human prostate carcinoma cell line (Du-145). Our purpose is to test the hypothesis that diverse human cancer cell lines, according to their adenosine receptor subclass status, would show differential growth modulation and that this will help tumor growth inhibition.

The reverse transcriptase–polymerase chain reaction assay (RT–PCR), which is based on the amplification of cell specific mRNAs, has been used to detect adenosine receptors. There are several studies in the literature that have evaluated the expression of adenosine receptors in different cancer cell lines by the RT-PCR technique [1-2]. Here we showed the detectable amounts of mRNA specific for A_1, A_{2A} and A_{2B} adenosine receptors.

Despite prostate cancer being the second leading cause of cancer death, little is known about the effects of nucleosides and nucleotides on the prostate or prostatic cell lines. Previously it was reported that ATP and related nucleotides activates P2Y or P2X purinergic receptors resulting in the stimulation of DU-145 prostate cancer cell proliferation [12]. Inconsistent with this paper Janssens and Boeynaems showed that ATP inhibited the growth of PC-3 and DU-145 prostate carcinoma cell lines [13].Some part of these effects was not entirely due to ATP, because the action of ecto-nucleotidases may shortened the ATP stability and converted it to adenosine [14], which is more stable than ATP, resulting in the activation of the adenosine receptors and their subsequent effects on cell proliferation.

The low level expression of A_3 receptor in most normal tissues versus its massive expression in different cancer cell lines (such as colon ,breast, melanoma, lymphoma, prostate) suggest this receptor as a target for cancer therapy [1-2].The high A_3 adenosine receptor mRNA expression level is found in colon and breast tumor versus adjacent normal tissues[15]. In colon cancer cell lines endogenous adenosine, through the interaction with A_3 receptors, mediates a tonic proliferative effect [16].We have further shown that the antiproliferative effects of adenosine in the human breast cancer cell lines were mediated via the A_3 receptor [4, 17]. Others suggested that IB-MECA, a synthetic A_3AR agonist, inhibits the growth of the human androgen-independent PC-3 prostatic carcinoma cell line [18],

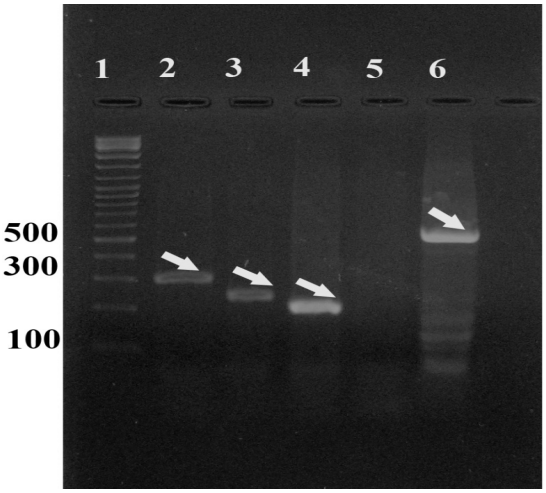

Figure1A. Expression of mRNA of adenosine receptors in the human lung adenocarcinoma cell line (Calu-6). mRNA was isolated and RT-PCR was performed as described in Materials and Methods. Experiments were repeated several times with identical results. Lane 1; 100-bp DNA ladder, Lane 2; A_1 (300 bp), lane 3; A_{2A} (237 bp), lane 4; A_{2B} (206 bp), lane 5; A_3, lane 6; beta actin(553 bp).

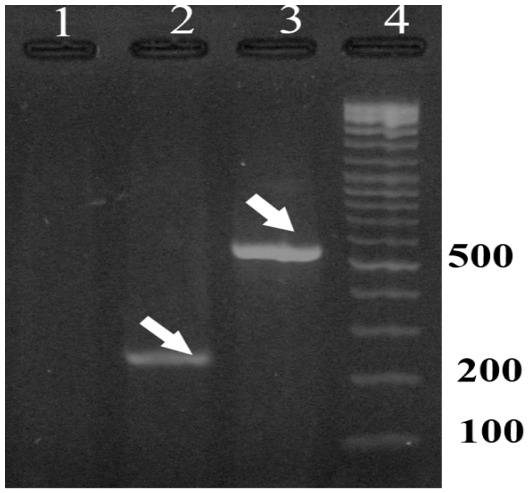

Figure1B. Expression of mRNA of adenosine receptors in the human lung adenocarcinoma cell line (Calu-6). mRNA was isolated and RT-PCR was performed as described in Materials and Methods. Experiments were repeated several times with identical results. Lane 1; A_3 (new primers: 156 bp), Lane 2; A_{2A} (237 bp), lane 3; beta-actin (553 bp), lane 4; 100-bp DNA ladder.

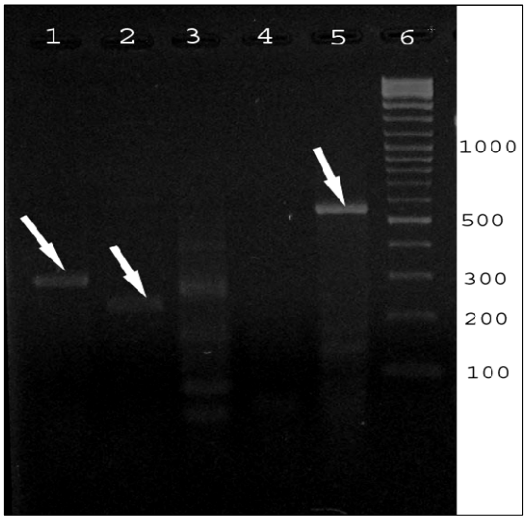

Figure2A. Expression of mRNA of adenosine receptors in the human prostate carcinoma cell line (Du-145). mRNA was isolated and RT-PCR was performed as described in Materials and Methods. Experiments were repeated several times with identical results. Lane 1; A_1 (300 bp), lane 2; A_{2A} (237 bp), lane 3; A_{2B} (483 bp), lane4; A_3, lane 5; beta-actin (553 bp), lane 6; 100-bp DNA ladder.

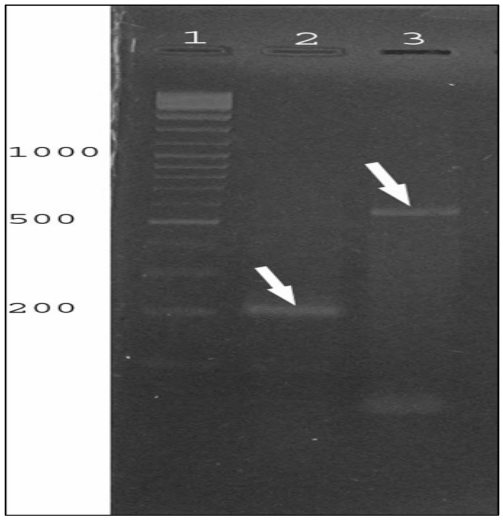

Figure2B. Expression of mRNA of adenosine receptors in the human prostate carcinoma cell line (Du-145). mRNA was isolated and RT-PCR was performed as described in Materials and Methods. Experiments were repeated several times with identical results. Lane 1; 100-bp DNA ladder, lane 2; A_{2B} (206 bp), lane 3; beta-actin (553 bp).

LNCaP human prostate adenocarcinoma and human colon adenocarcinoma cell line [19].Interestingly, we didn't find any expression for A_3 receptor in these two cell lines. This finding may be of importance because of inconsistent with previous attest on the role for A_3 in other cancers [2].

In another study Preston et al showed the presence of functional form of A_1 and A_{2A} adenosine receptors

on human cultured prostatic stromal cell (HCPSC) by examining their effects on cAMP production. These findings are consistent with a role for adenosine receptors in the modulation of adrenoceptor-mediated contractility in human prostate- derived cells [20].

From another point of view A_3 receptor showed the protective effects in ischemia/reperfusion-induced lung injury [21]. Using real-time RT-PCR and immunofluorescence Zhong et al demonstrated the expression of A_{2A}, A_{2B}, and A_3 adenosine receptors in murine lung mast cells, with a major role for A_3 receptor in mast cell degranulation [22].

In conclusion the results from this preliminary work indicates for the first time that Calu-6 and Du-145 cell lines exhibited adenosine receptors mRNA at a detectable level. Nevertheless, PCR and RT-PCR techniques did not allow a quantitative conclusion about the gene expression level. A comprehensive molecular, functional and biochemical study to clarify the effects of adenosine receptors on cell growth and development of different prostate cancer cell lines is in progress. Further work(s) will be required to confirm the significance of our findings and the functional effects of expressed adenosine receptors in the human lung cancer cells growth and development.

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Conflict of Interest

The authors have no conflict of interest in this article.

Authors' Contribution

PM designed the study, analyzed the data and wrote the paper. EB and YA participated in the acquisition, analysis and interpretation of data (as their Pharm.D. thesis research). MA and SH were involved in this study as co-supervisors of the thesis. All authors read and approved the final manuscript.

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