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Role of phosphodiesterase 2 in growth and invasion of human malignant melanoma cells

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ABSTRACT

Cyclic nucleotide phosphodiesterases (PDEs) regulate the intracellular concentrations and effects of adenosine 3′,5′-cyclic monophosphate (cAMP) and guanosine 3′,5′-cyclic monophosphate (cGMP). The role of PDEs in malignant tumor cells is still uncertain. The role of PDEs, especially PDE2, in human malignant melanoma PMP cell line was examined in this study. In PMP cells, 8-bromo-cAMP, a cAMP analog, inhibited cell growth and invasion. However, 8-bromo-cGMP, a cGMP analog, had little or no effect. PDE2 and PDE4, but not PDE3, were expressed in PMP cells. Growth and invasion of PMP cells were inhibited by erythro-9-(2-hydroxy-3-nonyl) adenine (EHNA), a specific PDE2 inhibitor, but not by rolipram, a specific PDE4 inhibitor. Moreover, cell growth and invasion were inhibited by transfection of small interfering RNAs (siRNAs) specific for PDE2A and a catalytically-dead mutant of PDE2A. After treating cells with EHNA or rolipram, intracellular cAMP concentrations were increased. Growth and invasion were stimulated by PKA14-22, a PKA inhibitor, and inhibited by N^6-benzoyl-c AMP, a PKA specific cAMP analog, whereas 8-(4-chlorophenylthio)-2′-O-methyl-cAMP, an Epac specific cAMP analog, did not. Invasion, but not growth, was stimulated by A-kinase anchor protein (AKAP) St-Ht31 inhibitory peptide. Based on these results, PDE2 appears to play an important role in growth and invasion of the human malignant melanoma PMP cell line. Selectively suppressing PDE2 might possibly inhibit growth and invasion of other malignant tumor cell lines.

1. Introduction

Cyclic nucleotide phosphodiesterases (PDEs) play an important role in signal transduction by modulating intracellular levels of cyclic nucleotides. These ubiquitous enzymes lower the intracellular concentrations of cyclic nucleotides by hydrolyzing adenosine 3′,5′-cyclic monophosphate (cAMP) and guanosine 3′,5′-cyclic monophosphate (cGMP) to their respective 5′nucleoside monophosphates [1]. The PDE superfamily represents 11 gene families (PDE1 to PDE11), which differ in their biochemical properties, their regulation, and their sensitivity to pharmacological agents [2]. PDE inhibitors affect many pathological conditions, but their use as anticancer agents has not been completely developed.

cGMP-stimulated phosphodiesterase (PDE2), a homodimer of two 105-kDa subunits, is found in association with intracellular membranes as well as in cytosolic fractions. PDE2 enzymatic activity was first described in rat liver extracts [3] and was subsequently purified to apparent homogeneity from various tissues, including bovine heart and adrenal gland [4], calf liver [5], bovine brain [6], rat liver [7], and rabbit brain [8]. Although cGMP is the preferred substrate and effector molecule for this enzyme, PDE2 hydrolyzes both cGMP and cAMP with positively cooperative kinetics. At physiological concentrations of cyclic nucleotides, PDE2 responds to elevated cGMP with increased hydrolysis of cAMP. Therefore, PDE2, which is sometimes referred to as a cGMP-stimulated cAMP PDE, can provide crosstalk between cAMP and cGMP signaling pathways [9].

cAMP is a positive intracellular signal for cell proliferation in many differentiated cells [10,11]. In many tumor cells, however, cAMP is a negative messenger for proliferation, with lower basal cAMP concentrations in some tumor cells than in normal cells [11]. Various agents that increase cAMP have previously been found to inhibit tumor cell growth

Abbreviations: cAMP, adenosine 3′,5′-cyclic monophosphate; cGMP, guanosine 3′,5′-cyclic monophosphate; dnPDE2A, a catalytically-dead mutant of PDE2; EHNA, erythro-9-(2-hydroxy-3-nonyl)adenine; FBS, fetal bovine serum; MTS, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium; PBS, phosphate-buffered saline; PDE, phosphodiesterase; RT-PCR, reverse transcription-polymerase chain reaction; siRNA, small interfering RNA; 8-Br-cAMP, 8-bromoadenosine 3′,5′-cyclic monophosphate; 8-Br-cGMP, 8-bromoguanosine 3′,5′-cyclic monophosphate.

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Table 1
Sequences of specific primer pairs for PDE2, PDE3, and PDE4 used in RT-PCR analysis.

Gene	Sequence of primer pairs
PDE2A	5′-GCA TGT GTC ATG ACC TGG AC-3′
	5′-AGC ATG CGC TGA TAG TCC TT-3′
PDE3A	5′-TCA CCT CTC CAA GGG ACT CCT-3′
	5′-CAG CAT GTA AAA CAT CAG TGG C-3′
PDE3B	5′-AAT TCT TCC AAC CAT CGA CC-3′
	5′-GCA TGT AGC ACA TCT GTG GC-3′
PDE4A	5′-AAC AGC CTG AAC AAC TCT AAC-3′
	5′-CAA TAA AAC CCA CCT GAG ACT-3′
PDE4B	5′-AGC TCA TGA CCC AGA TAA GTG-3′
	5′-ATA ACC ATC TTC CTG AGT GTC-3′
PDE4C	5′-TCG ACA ACC AGA GGA CTT AGG-3′
	5′-GGA TAG AAG CCC AGG AGA AAG-3′
PDE4D	5′-CGG AGA TGA CTT GAT TGT GAC-3′
	5′-CGT TCC TGA AAA ATG GTG TGC-3′

in vitro. However, PDE inhibitors, especially those of the methylxanthine type, display growth inhibition only at rather high concentrations [11–18]. Elevation of intracellular cAMP levels can regulate the metastatic ability of tumor cells either positively [19–21] or negatively [22–25], depending upon the cell type.

cGMP regulates smooth muscle relaxation, platelet aggregation, and neurotransmission. It has been reported that increasing intracellular cGMP induces apoptosis in a colon tumor cell line [26]; however, it has also been reported that the cGMP analogs did not inhibit proliferation in a malignant glioma cell line [25].

cAMP and cGMP exist in all cells; by catalyzing hydrolysis of these second messengers, PDEs are thought to play important roles in regulation of their signals. However, the role of PDE2 is still unclear in malignant tumor cells. In this study, the role of PDE2 in growth and invasion of a malignant melanoma cell line was examined using a specific PDE2 inhibitor and PDE2A small interfering RNAs (siRNAs).

2. Materials and methods

2.1. Cell culture

Human malignant melanoma PMP cells [27] were established from a 65-year-old patient with primary palatal malignant melanoma who was treated in our department in 1986. PMP cells had a polygonal shape and were amelanotic. PMP cells were maintained in RPMI 1640 medium (Invitrogen, Carlsbad, CA) supplemented with 5% fetal bovine serum (FBS) (Sigma, St. Louis, MO). HMG cells (which expressed PDE3A, PDE3B and PDE4D, and were used as positive control for PDE3A, PDE3B and PDE4D RT-PCR) were maintained in RPMI 1640 medium supplemented with 10% FBS, and KB cells (which expressed PDE4A and were used as positive control for PDE4A immunoblots) were maintained in RPMI 1640 medium supplemented with 10% FBS at 37 °C in a humidified 5% CO_2 atmosphere.

2.2. Cell growth assays

Cells were plated at 400 cells/well in 96-well plates, allowed to adhere for 24 h, and then cultured in the absence or presence of different concentrations of reagents for 3 or 5 days. MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium] assays were performed using CellTiter 96 Aqueous One Solution Cell Proliferation Assay (Promega, Madison, WI), and cell numbers were calculated.

2.3. In vitro invasion assays

PMP cells (4×10^4 cells) in RPMI 1640 medium containing 0.1% FBS were transferred to 8 μm pore Matrigel pre-coated inserts (BD Bioscience, Bedford, MA). The inserts were placed in companion wells containing RPMI 1640 medium supplemented with 10% FBS as a

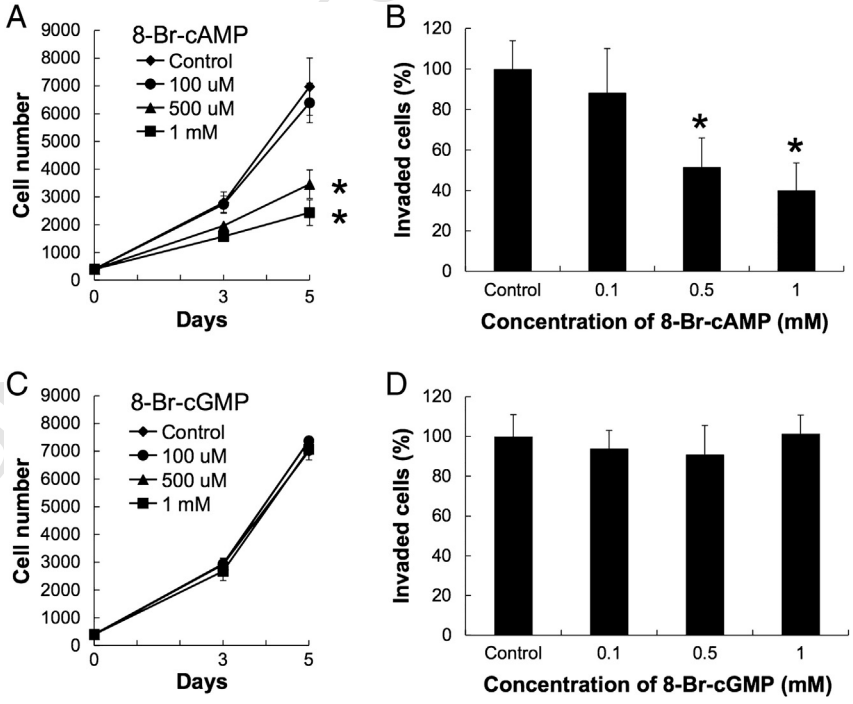

Fig. 1. Effects of 8-Br-cAMP or 8-Br-cGMP on cell growth and invasion. Cell growth was measured using the MTS assay. Cells were cultured in the absence or presence of 8-Br-cAMP (0.1 to 1 mM) or 8-Br-cGMP (0.1 to 1 mM) for 5 days. Cell invasion was examined by in vitro Matrigel invasion assays. Cells were transferred to 8 μm pore Matrigel pre-coated inserts, and 8-Br-cAMP (0.1 to 1 mM) or 8-Br-cGMP (0.1 t 1 mM) was added. After a 16 h incubation, invaded cells were stained with May-Grünwald–Giemsa stain and counted. Data in graphs are means of three independent experiments, each performed in duplicate. (A) Effect of 8-Br-cAMP on cell growth. (B) Effect of 8-Br-cAMP on cell invasion. (C) Effect of 8-Br-cGMP on cell growth. (D) Effect of 8-Br-cGMP on cell invasion. The error bars represent means ± SD, $n = 3$. The treatments that differ significantly from control are noted (*, $P < 0.01$).