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Involvement of Src and the actin cytoskeleton in the antitumorigenic action of adenosine dialdehyde

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ABSTRACT

Transmethylation is an important reaction that transfers a methyl group in *S*-adenosylmethionine (SAM) to substrates such as DNA, RNA, and proteins. It is known that transmethylation plays critical roles in various cellular responses. In this study, we examined the effects of transmethylation on tumorigenic responses and its regulatory mechanism using an upregulation strategy of adenosylhomocysteine (SAH) acting as a negative feedback inhibitor. Treatment with adenosine dialdehyde (AdOx), an inhibitor of transmethylation-suppressive adenosylhomocysteine (SAH) hydrolase (SAHH), enhanced the level of SAH and effectively blocked the proliferation, migration, and invasion of cancer cells; the treatment also induced the differentiation of C6 glioma cells and suppressed the neovascular genesis of eggs in a dose-dependent manner. Through immunoblotting analysis, it was found that AdOx was capable of indirectly diminishing the phosphorylation of oncogenic Src and its kinase activity. Interestingly, AdOx disrupted actin cytoskeleton structures, leading to morphological changes, and suppressed the formation of a signaling complex composed of Src and p85/PI3K, which is linked to various tumorigenic responses. In agreement with these data, the exogenous treatment of SAH or inhibition of SAHH by specific siRNA or another type of inhibitor, 3-deazaadenosine (DAZA), similarly resulted in antitumorigenic responses, suppressive activity on Src, the alteration of actin cytoskeleton, and a change of the colocalization pattern between actin and Src. Taken together, these results suggest that SAH/SAHH-mediated transmethylation could be linked to the tumorigenic processes through cross-regulation between the actin cytoskeleton and Src kinase activity.

1. Introduction

Transmethylation (or methylation) is a biochemical modification that involves the transfer of a methyl group from *S*-adenosylmethionine (SAM) to various methyl-accepting proteins, DNA, and RNA molecules [1,2]. The critical importance of this process has been implicated in cancer, aging, obesity, and Parkinson's disease; however, the pathophysiological mechanism of methylation in each of these processes has not been fully elucidated [3–6]. Transmethylation involves different enzymes, including *S*-adenosylhomocysteine hydrolase (SAHH), methionine methyltransferase, and protein/DNA/RNA methyltransferases (*N*-, *O*-, and *S*-methyltransferases) [7–9]. In addition, inhibitors such as adenosine dialdehyde (AdOx), 3-deazaadenosine, and 5-aza-2′-deoxycytidine can block these enzymes and suppress transmethylation [10].

Oncogenic tyrosine kinases are highly active in many types of cancer cells with enhanced capacity for proliferation, survival, and metastasis [11]. One such tyrosine kinase is Src, which contains SH2 and SH3 domains and is the prototypical member of the Src family kinases (SFKs) that also include Lck, Hck, Fyn, Blk, Lyn, Fgr, Yes, and Yrk [12]. Indeed, Src has been shown to perform multiple functions in tumorigenesis for a variety of cancers, including small cell lung cancer, ovarian cancer, oral squamous cell carcinoma, hepatocytoma, and prostate cancer [13–15]. These roles include the regulation of proliferation, differentiation, invasion, metastasis, angiogenesis, and motility of tumor

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cells [16]. In light of these data, much attention has been focused on the development of anti-cancer drugs that target oncogenic Src kinase [17,18].

Although several reports have suggested that transmethylation is an important reaction in the cancerous state, the exact regulatory mechanism of the proliferation, differentiation, adhesion, invasion, and migration of tumor cells in terms of target cytosolic proteins is not fully understood. Obviously, understanding the regulatory mode of action of transmethylation can clearly lead us to find a novel anti-cancer drug target. Interestingly, it has been reported that histidine methylation at histidine 73 (His-73) residue of actin plays a critical role in actin polymerization [19,20], requiring for intracellular signaling, contractile ring formation, and cell migration [21]. Src has been also reported to modulate such cellular responses via modulating actin polymerization [22,23], implying that there might be a cross-regulation between Src and actin polymerization by transmethylation. Therefore, the aim of this study was to explore the potential role of the transmethylation event in regulating Src kinase activation, responsible for tumorigenic responses, in terms of actin polymerization. To do this, blockade of transmethylation by broad spectrum methyltransferase inhibitory AdOx and siRNA to SAHH was employed to prove this hypothesis.

2. Materials and methods

2.1. Materials

S-adenosyl-L-[methyl-^{3}H] methionine (85 Ci/mmol) was purchased from Amersham Pharmacia Biotech (Buckinghamshire, UK). Rat C6 glioma, SYF, HeLa, HEK293, L929, A431, MCF-7, MDA-MB-231, and HT-29 cells were purchased from the American Type Culture Collection (Manassas, VA). TLR4-expressing HEK293 cells were purchased from Invitrogen (San Diego, CA). GF109203X, KT5720, PP2, piceatannol, LY294002, and AMI-1 were purchased from Calbiochem (La Jolla, CA). Jasplakinolide (Jasp), cytochalasin B (Cyto B), latrunculin B (Lat B), chloroquine, genistein, 3-deazaadenosine (DAZA), all-*trans* retinoic acid (ATRA), *S*-adenosylhomocysteine (SAH), adenosine dialdehyde (AdOx), and porbal-12-myristate-13-acetate (PMA) were purchased from Sigma Chemical Co. (St. Louis, MO). Antibodies to phospho- and total forms of Src (Y416/Y527), p85, metalloproteinase (MMP)-9, G-actin, IκBα c-Fgr, c-Yes, Hck, Fyn, Lyn, p21$^{WAF/CIP}$, p27^{kip1}, HA, *S*-adenosylhomocysteine hydrolase (SAHH), green florescence protein (GFP), histone deacetylase (HDAC), histone H3, and β-actin were purchased from Cell Signaling (Beverly, MA) or Santa Cruz Biotechnology (Santa Cruz, CA). siRNA to SAHH was obtained from Santa Cruz Biotechnology (Santa Cruz, CA).

2.2. Construction of expression vectors

A GFP-fused wild-type actin construct (GFP-Actin-WT) was prepared via amplification with a typical culture method using competent *E. coli* (DH5α). The pcDNA-HA, pcDNA-HA-tagged c-Src construct (Src-WT), Src Lys295 mutants (kinase-deficient HA-Src, Src-KD), and Src deletion mutants (Src-ΔSH2 and Src-ΔSH3) used in this study were the same as reported previously [24].

2.3. Cell culture

Rat glioma C6, HEK293, TLR4-expressing HEK293, HeLa, L929, A431, MDA-MB-231 (MDA), HT-29, RAW264.7, and peritoneal macrophage cells were cultured in Dulbecco's modified Eagle's medium (DMEM) or RPMI1640 with 10% fetal bovine serum and 100 U/ml penicillin/streptomycin at 37 °C in a humidified atmosphere containing 5% CO_2.

2.4. Determination of tumorigenic responses

For the cell proliferation assay, cell viability was assessed by conventional MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay [25,26]. For the wound healing (cell migration) assay, HeLa cells were grown to a confluent monolayer in 60 mm plates. A wound was introduced as previously described by scraping the monolayer with a p200 pipette tip [27,28]. Phase contrast images were acquired with an inverted microscope (Olympus, Tokyo, Japan). For the invasion assay, the invasive capacity of MDA-MB-231 cells was measured as reported previously by using matrigel-coated plates [29,30]. After incubation with the appropriate drug for 24 h, the cells were fixed in 4% formaldehyde; subsequently, hematoxylin and eosin staining was used to count the number of cells that successfully penetrated the matrigel layer. The effect on angiogenesis was assessed as reported previously via the chorioallantoic membrane (CAM) assay [29].

2.5. Reverse transcription and real-time polymerase chain reaction (PCR)

C6 cells were plated at a density of 1.0×10^5 cells/well in 6-well tissue culture plates. After treatment with the indicated reagents, total RNA was extracted with TRIZOL (GIBCO-BRL, Grand Island, NY, USA). After measuring the total amount of RNA and electrophoresis in a formaldehyde-agarose gel, cDNAs were synthesized with MMLV RTase (SuperBio, Daejeon, Korea). Semiquantitative polymerase chain reaction (PCR) and real-time PCR were performed as previously described [31,32].

2.6. Confocal microscopy

The confocal microscopic analysis of actin, Src, vimentin, GFAP, and nuclei were performed on C6 glioma, HeLa or HEK293 cells as previously described [33].

Table 1
List of primers or siRNA sequences used in this study.

mRNA		Primer sequences
Semiquantitative		
p21$^{WAF/CIP}$	F	5′-TGGACAGTGAGCAGTTGAGC-3′
	R	5′-GAGTGCAAGACAGCGACAAG-3′
p27^{kip1}	F	5′- ATTTCGACTTTCAGAATCATAAGCC-3′
	R	5′-CTGAAACATTTTCTTCTGTTCTGCT-3′
GFAP	F	5′-ATGCAAGAAACAGAAGAGTGGTATC-3′
	R	5′-GCTTAACGTTGAGTAGATCCTGGTA-3′
Vimentin	F	5′-GTCATTCAGACAGGATGTTGACAAt-3′
	R	5′-ATCTCTTCCTTCATGTTCTGGATCT-3′
hSAHH	F	5′-CGTCATCATCACCGAGATTG-3′
	R	5′-CCACGTCAAAGTGTCCAATG-3′
hMAT1a	F	5′-GGTGTCACTGGCCGTAAGAT-3′
	R	5′-GGCATAGGAAACCTGGACAA-3′
hMAT2a	F	5′-TTCCCATCAGAGTCCACACA-3′
	R	5′-ATTTTGCGTCCAGTCAAACC-3′
hMAT2b	F	5′-AATTGCAGATGCCTTCAACC-3′
	R	5′-GTCAATGAGGAAAGGCCAAA-3′
Quantitative (Real-time)		
p21$^{WAF/CIP}$	F	5′-ATTTCTATCACTCCAAGCG-3′
	R	5′-ACACTGAATGAAGGCTAAGG-3′
p27^{kip1}	F	5′-AGTGTCCTTTCGGTGAGA-3′
	R	5′-TACATAACAGAATCTTCGGAACTC-3′
hSAHH	F	5′-TGAGAAAGAACAGAGAAGTGA-3′
	R	5′-CCAGGGTTGTGAAAGGAA-3′
Target protein		siRNA sequences
Control	Sense	5′-CCUACGCCACCAAUUUCGU-3′
	Antisense	5′-ACGAAAUUGGUGGCGUAGG-3′
SAHH	Sense	5′-CUGACAAACUGCCCUACAA-3′
	Antisense	5′-UUGUAGGGCAGUUUGUCA-3′