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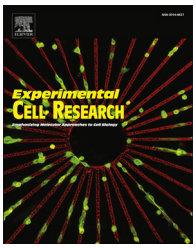

Research Article

Polyamine and methionine adenosyltransferase 2A crosstalk in human colon and liver cancer

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ABSTRACT

Methionine adenosyltransferase (MAT) is an essential enzyme that is responsible for the biosynthesis of S-adenosylmethionine (SAMe), the principal methyl donor and precursor of polyamines. *MAT1A* is expressed in normal liver and *MAT2A* is expressed in all extrahepatic tissues. *MAT2A* expression is increased in human colon cancer and in colon cancer cells treated with mitogens, whereas silencing *MAT2A* resulted in apoptosis. The aim of the current work was to examine the mechanism responsible for *MAT2A*-dependent growth and apoptosis. We found that in RKO (human adenocarcinoma cell line) cells, *MAT2A* siRNA treatment lowered cellular SAMe and putrescine levels by 70–75%, increased apoptosis and inhibited growth. Putrescine supplementation blunted significantly *MAT2A* siRNA-induced apoptosis and growth suppression. Putrescine treatment (100 pmol/L) raised *MAT2A* mRNA level to 4.3-fold of control, increased the expression of c-Jun and c-Fos and binding to an AP-1 site in the human *MAT2A* promoter and the promoter activity. In human colon cancer specimens, the expression levels of MAT2A, ornithine decarboxylase (ODC), c-Jun and c-Fos are all elevated as compared to adjacent non-tumorous tissues. Overexpression of ODC in RKO cells also raised MAT2A mRNA level and MAT2A promoter activity. ODC and MAT2A are also overexpressed in liver cancer and consistently, similar MAT2A-ODC-putrescine interactions and effects on growth and apoptosis were observed in HepG2 cells. In conclusion, there is a crosstalk between polyamines and MAT2A. Increased *MAT2A* expression

Abbreviations: AP-1, activator protein-1; BrdU, 5-bromo-2′-deoxyuridine; ChIP, chromatin immunoprecipitation; dcSAMe, decarboxylated SAMe; EGF, epidermal growth factor; HCC, hepatocellular carcinoma; HPLC, high-performance liquid chromatography; IGF-1, insulin-like growth factor-1; MAT, methionine adenosyltransferase; ODC, ornithine decarboxylase; PCR, polymerase chain reaction; SAMe, S-adenosylmethionine; Sc, scrambled siRNA; siRNA, small interfering RNA

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provides more SAMe for polyamines biosynthesis; increased polyamine (putrescine in this case) can activate *MAT2A* at the transcriptional level. This along with increased ODC expression in cancer all feed forward to further enhance the proliferative capacity of the cancer cell.

Introduction

Colorectal cancer is a major cause of morbidity and mortality as the third most common cancer worldwide and the fourth most common cause of cancer death [1]. In the past 20 years, tremendous progress has been made on understanding the molecular pathogenesis of this common cancer. Four signaling pathways commonly aberrant in colorectal cancer are: (1) Wnt, (2) K-ras, (3) transforming growth factor β, and (4) *p*53 [2]. Mutations in these pathways can lead to inactivation of tumor suppressor function and/or activation of proto-oncogenes. In addition, aberrant signaling in several growth factors such as insulin-like growth factor receptor-1 (IGF-1R), epidermal growth factor receptor (EGFR) and leptin has also been shown to be important in colon cancer growth, invasion and metastasis [3–6].

Methionine adenosyltransferase (MAT) is an essential enzyme that catalyzes the formation of S-adenosylmethionine (SAMe), the principal biological methyl donor and precursor for polyamine biosynthesis [7]. Two genes encode for the catalytic subunit of MAT, *MAT1A* encodes for α1 that is expressed predominantly by normal liver as dimer (MATIII) and tetramer (MATI), and *MAT2A* encodes for α2 that is expressed by all extrahepatic tissues as the MATII isoenzyme [7]. We reported that *MAT2A* expression is induced in human colorectal cancer and in colon cancer cells treated with IGF-1, EGF, and leptin [8]. Importantly, mitogenic effect of these growth factors was abolished if *MAT2A* induction was prevented [8]. In addition, knockdown of *MAT2A* by siRNA in colon cancer cells resulted in increased apoptosis [8]. Thus, *MAT2A* expression is a key factor that regulates growth and apoptosis in colon cancer cells.

Given the importance of SAMe in polyamine biosynthesis, a logical hypothesis is that increased *MAT2A* expression would provide an increased supply of SAMe to enhance polyamine biosynthesis. Polyamines are organic cations with multiple functions that are essential for the cell's survival [9]. Mammalian cells have three polyamines: putrescine, spermidine and spermine. They interact with DNA (causing changes in chromatin structure), RNA and proteins and have antioxidant properties [10,11]. They regulate cell cycle progression and inhibition of polyamine synthesis leads to cell cycle arrest and apoptosis [10]. Of interest, the expression of ornithine decarboxylase (ODC), the rate-limiting enzyme responsible for the initial step in polyamine biosynthesis, namely formation of putrescine, is often induced and polyamine levels are higher in colon cancer [11]. However, whether MAT2A regulates growth and apoptosis via polyamines has not been examined. In this work we uncovered a crosstalk between MAT2A and polyamines that has not been previously recognized which can further enhance the growth of the cancer cell.

Materials and methods

Materials

Antibodies used for Western blot analyses of MATII and c-Jun were purchased from Santa Cruz Biotechnology (Santa Cruz, CA), ODC was from Abcam (Cambridge, MA) and β-actin was from Sigma–Aldrich (St. Louis, MO). All other reagents were of analytical grade and obtained from commercial sources.

Cell culture and putrescine treatment

RKO and HepG2 cells were obtained from the Cell Culture Core of the USC Research Center for Liver Diseases at Keck School of Medicine. Cells were grown according to instructions provided by American Type Cell Collection (Rockville, MD). RKO and HepG2 cells were grown to 50–60% confluence on six-well plates, and changed to media containing 1% for 24 h before treatment with 50–200 pmol/L or 50–200 μmol/L of putrescine (Sigma), respectively, for 24 h.

Tissue specimens

Thirteen colon cancers and adjacent non-tumorous tissues were obtained by Prof. Giordano (Whipps Cross University Hospital, London, UK) during surgical resection for primary colon cancer. These tissues were immediately frozen in liquid nitrogen for subsequent RNA and protein extraction. Written informed consent was obtained from each patient. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a prior approval by Keck School of Medicine University of Southern California's human research review committee.

RNA extraction and real-time polymerase chain reaction (PCR) analysis

Total RNA isolated from cells and colons as described [8] was subjected to reverse transcription by using M-MLV Reverse Transcriptase (Invitrogen, Carlsbad, CA). One microliter of reverse transcription product was subjected to quantitative real-time PCR analysis. The primers and TaqMan probes for *MAT2A, ODC*, *c-Jun*, *c-Fos,* and Universal PCR Master Mix were purchased from ABI (Foster City, CA). Hypoxanthine phosphoribosyl-transferase 1 and 18S rRNA were used as housekeeping genes as described [8]. The delta Ct (ΔCt) obtained was used to find the relative expression of genes according to the following formula: relative expression$=2^{-\Delta\Delta Ct}$, where $\Delta\Delta Ct=\Delta Ct$ of respective genes in experimental groups $-\Delta Ct$ of the same genes in control group.

RNA interference and overexpression experiments

The pre-designed small interfering RNA (siRNA) targeting human *MAT2A* (sense sequence: ACACAUUGGAUAUGAUGAUTT) (Invitrogen, Carlsbad, CA) was as described [8], *ODC* (sc-43982, Santa Cruz, CA) and negative control siRNA (Ambion, Austin, TX) were purchased commercially. RKO and HepG2 cells were cultured in six-well plate (3×10^5 cells/well) and transfected using RNAiMax (5 μL/well) from Invitrogen with MAT2A, ODC siRNA (10 nM)