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Hydrogen peroxide triggers a novel alternative splicing of arsenic (+3 oxidation state) methyltransferase gene

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ABSTRACT

We previously reported that two splicing variants of human AS3MT mRNA, exon-3 skipping form (Δ3) and exons-4 and -5 skipping form (Δ4,5), were detected in HepG2 cells and that both variants lacked arsenic methylation activity (Sumi et al., 2011). Here we studied whether hydrogen peroxide (H_2O_2) triggers alternative splicing of AS3MT mRNA. The results showed that exposure of HepG2 cells to H_2O_2 resulted in increased levels of a novel spliced form skipping exon-3 to exon-10 (Δ3-10) in an H_2O_2-concentration-dependent manner, although no change was detected in the mRNA levels of Δ3 AS3MT. We found decreased protein levels of serine/arginine-rich 40 (SRp40), which we determined to be a candidate splice factor for controlling the splicing of AS3MT mRNA. We next compared the amounts of methylated arsenic metabolites between control and H_2O_2-exposed HepG2 cells after the addition of arsenite as a substance. The results showed lower levels of methylated arsenic metabolites in HepG2 cells exposed to H_2O_2. These data suggest that the splicing of AS3MT pre-mRNA was disconcerted by oxidative stress and that abnormal alternative splicing of AS3MT mRNA may affect arsenic methylation ability.

1. Introduction

In mammals, arsenic (+3 oxidation state) methyltransferase (AS3MT) catalyzes the formation of monomethylarsonate (MMA(V)) and dimethylarsinic acid (DMA(V)) from arsenite (As(III)) and monomethylarsonous acid (MMA(III)), respectively, as a substrate [1–4]. Arsenic methylation has been considered a detoxification process of inorganic arsenicals, although studies have indicated that MMA(III) and DMA(III), the intermediate products of arsenic methylation, are more toxic than inorganic arsenicals [5–7]. We previously found two splicing variants of the human AS3MT mRNA: exon-3 skipping form (Δ3) and exons-4 and -5 skipping form (Δ4,5) [8]. When we compared the methyltransferase activity of wild-type and Δ4,5 AS3MT recombinant proteins, Δ4,5 AS3MT protein showed no arsenic methyltransferase activity [8]. These data suggest that the splicing variants of the AS3MT gene may affect the capacity of arsenic methylation in cells.

The splicing of pre-mRNA, the removal of introns and concomitant joining of exons, is regulated by the spliceosome. As many as 150 associated proteins are involved in the spliceosome, and the assembly of these proteins can be promoted by positive-acting factors such as serine/arginine (SR)-rich proteins and inhibited by negative-acting factors such as heterogeneous nuclear ribonucleoproteins (hnRNP) [9,10]. Alternative splicing of pre-mRNA transcripts is a source of protein diversity and has been involved in the onset of several diseases, including cancer and Parkinson's disease [11–14]. Environmental chemicals that are known to cause oxidative stress, such as paraquat and arsenic, were shown to impair control over mRNA splicing, resulting in the deregulation of the survival of motor neurons (SMN) and the induction of DNA damage in gene 45α (GADD45α) [15,16]. In addition, it has been reported that hydrogen peroxide (H_2O_2) stimulates alternative splicing of hypoxanthine guanine phosphoribosyl transferase (HPRT), of DNA polymerase β (POLB), and of soluble guanylyl cyclase (sGC) [17,18]. Thus, it is apparent that oxidative stress causes splicing abnormalities on specific mRNAs. However, it remains unknown whether the control of splicing of AS3MT mRNA is vulnerable to oxidative stress.

In this study, we found a novel splicing variant of AS3MT mRNA in HepG2 cells that was triggered by H_2O_2. SRp40, a candidate

Abbreviations: As(III), inorganic arsenite; DMA(V), dimethylarsinic acid; HPLC-ICP-MS, high-performance liquid chromatography–inductively coupled plasma mass spectrometry; MMA(V), monomethylarsonate.

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factor for the control of splicing in AS3MT mRNA, was decreased by H_2O_2 through the ubiquitin-proteasome pathway. In addition, arsenic methylation activity in HepG2 cells was decreased by exposure to H_2O_2.

2. Materials and methods

2.1. Materials

Hydrogen peroxide was purchased from Wako Pure Chemical Industries (Osaka, Japan). MG-132 was purchased from Cell Signaling Technology (Danvers, MA). Antibodies for SR and Lamin B were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Monomethylarsonate (MMA(V)) was purchased from Tri Chemical Laboratories (Yamanashi, Japan). All other reagents and chemicals used were of the highest grade available.

2.2. Cell culture

HepG2 cells were obtained from ATCC (Manassas, VA, USA) and were cultured at 37 °C in a humidified atmosphere of 5% CO_2 using Dulbecco's modified Eagle's medium (Wako Pure Chemical) containing 10% fetal calf serum, penicillin (100 U/ml), and streptomycin (100 μg/ml).

2.3. RT-PCR

The total RNA was isolated with ISOGEN (Nippon Gene, Toyama, Japan). The RT-PCR experiments were conducted with a SuperScript III One-Step RT-PCR system with Platinum Taq DNA polymerase (Invitrogen, Carlsbad, CA). The reaction mixture was incubated at 45 °C for 30 min for reverse transcription. PCR reactions were then carried out according to the following protocol: 1 cycle of 94 °C for 2 min followed by 35 cycles of 94 °C for 15 s, 55 °C for 30 s, and 68 °C for 2 min, and a final cycle at 68 °C for 5 min. The primer sequences are shown in Table 1. The amplified products were resolved by 1% agarose gel electrophoresis. The bands of WT and of Δ3 and Δ3-9 AS3MT with the combination of AS3MT(WT)-f and AS3MT(WT)−r primers were detected at 1,083, 955 and 240 bp, respectively. The band of Δ3-9 AS3MT with the combination of AS3MT(Δ3-9)-f and AS3MT(WT)−r primers was detected at 222 bp.

2.4. Cloning of AS3MT spliced form

Amplified cDNA (240 bp in Fig. 1) was extracted from agarose gel with the QIAquick Gel Extraction Kit (Qiagen, Valencia, CA) and was cloned using a TOPO TA cloning Kit (Invitrogen) to determine the DNA sequence.

2.5. Western blotting

The nuclear fraction was extracted with the NE-PER Nuclear and Cytoplasmic Extraction Reagent Kit (Thermo Scientific, Waltham, MA). Samples for each analysis were separated by SDS-PAGE. Gels were transferred to an immune-blot PVDF membrane and then placed in a blocking solution consisting of TBST (10 mM Tris (pH 8.0), 150 mM NaCl, and 0.05% Tween 20) and 5% skim milk for 1 h. The blotted membranes were incubated with SR antibody, washed with TBST, and incubated with HRP-conjugated secondary antibody. Bound IgG was visualized with Western Blotting Detection Reagents (Thermo Scientific) according to the manufacturer's protocol.

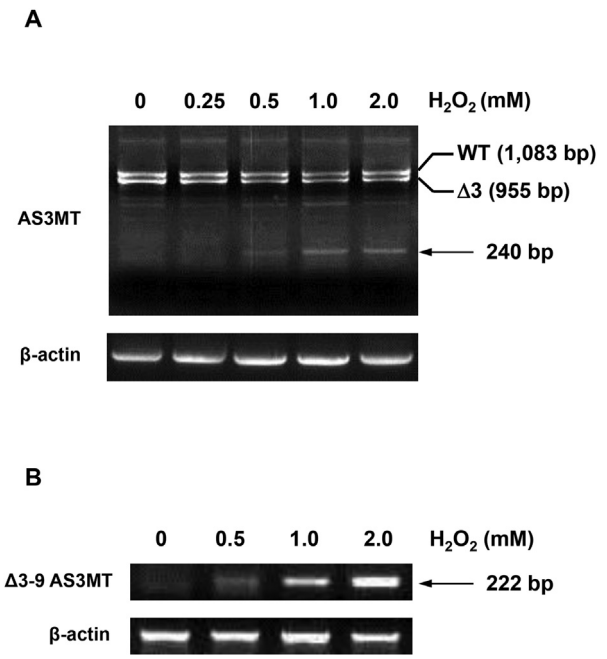

Fig. 1. A novel spliced form of human AS3MT mRNA triggered by H_2O_2 exposure. A and B: HepG2 cells were exposed to indicated concentrations of H_2O_2 for 6 h. The total RNA was extracted, and RT-PCR was performed using the primers of AS3MT (WT)-f and AS3MT (WT)−r, (A) and the primers of AS3MT(Δ3,9)-f and AS3MT (WT)−r, (B). β−actin was detected as a loading control. Representative data from three individual determinations are shown.

2.6. Determination of arsenic methylation activity

Arsenic speciation of cell lysates with HPLC-ICP-MS was measured as previously reported [8]. HepG2 cells were pre-incubated with 1 and 2 mM H_2O_2 for 3 h followed by exposure to 10 μM As(III) for 24 h. The cell lysates suspended in 100 μL of 150 mM Tris-HNO_3 were then sonicated and incubated at 70 °C for 30 min to inactivate cellular catalase, and H_2O_2 was added to a final concentration of 10% at room temperature for 3 h for the oxidation of arsenic metabolites. Each of these samples was centrifuged at 15,000 × *g* for 10 min at 4 °C, and the supernatant was applied to an Amicon YM-3 centrifugal filter (Millipore, Billerica, MA) at 15,000 × *g* for 20 min at 4 °C. The eluate (5 μL) was separated by a Nanospace HPLC system (Shiseido, Tokyo, Japan) on a Capcell Pak C18 MGII (1.0 mm i.d. × 150 mm long) (Shiseido) using 5 mM tetrabutylammonium hydroxide, 3 mM malonic acid, and 4% methanol, as a mobile phase with a flow rate of 200 μL/min. The eluates from the HPLC column were directly introduced into the ICP-MS spray chamber via a PEEK tube (0.13 mm i.d.) (ICP-MS

Table 1
Primer sequences.

Name	Sequence	Located at
AS3MT (WT)−f	GACGCTGAGATACAGAAGGACGTGC	Exon 2
AS3MT (WT)−r	TCCAGCAGCATCAGGGACACA	Exon 11
AS3MT (Δ3−9)−f	GACGTGCAGGAAGGTGAAA	Exon 2 and 10 junction

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