FEBS Letters 587 (2013) 231–237

FEBS Letters

journal homepage: www.FEBSLetters.org

Sulfation of keratan sulfate proteoglycan reduces radiation-induced apoptosis in human Burkitt's lymphoma cell lines

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ARTICLE INFO

Article history:
Received 14 August 2012
Revised 18 October 2012
Accepted 3 December 2012
Available online 10 December 2012

Edited by Vladimir Skulachev

Keywords:
Apoptosis
Burkitt's lymphoma
Keratan sulfate
PAPST
Radiation

ABSTRACT

This study focuses on clarifying the contribution of sulfation to radiation-induced apoptosis in human Burkitt's lymphoma cell lines, using 3′-phosphoadenosine 5′-phosphosulfate transporters (PAPSTs). Overexpression of PAPST1 or PAPST2 reduced radiation-induced apoptosis in Namalwa cells, whereas the repression of PAPST1 expression enhanced apoptosis. Inhibition of PAPST slightly decreased keratan sulfate (KS) expression, so that depletion of KS significantly increased radiation-induced apoptosis. In addition, the repression of all three *N*-acetylglucosamine-6-*O*-sulfotransferases (CHST2, CHST6, and CHST7) increased apoptosis. In contrast, PAPST1 expression promoted the phosphorylation of p38 MAPK and Akt in irradiated Namalwa cells. These findings suggest that 6-*O*-sulfation of GlcNAc residues in KS reduces radiation-induced apoptosis of human Burkitt's lymphoma cells.

1. Introduction

Sulfation of a variety of molecules, including glycoproteins, proteoglycans (PGs), and glycolipids, is an important posttranslational modification, which modifies the properties of molecules by conferring a negative charge. Heparan sulfate (HS) and chondroitin sulfate (CS) have highly-sulfated glycosaminoglycan (GAG) chains, which play important roles in many biological processes. For example, a number of fibroblast growth factors (FGFs) interact with these sulfated GAG subtypes [1], so that inhibition of sulfation was able to reduce the signaling of FGF. Therefore, the sulfation of PGs may regulate numerous physiological and pathological events.

The process of sulfation requires the involvement of a high energy form of the universal sulfate donor, namely, 3′-phosphoadenosine 5′-phosphosulfate (PAPS). In higher organisms, PAPS is synthesized in the cytosol or nucleus by PAPS synthetases [2], and PAPS transporters (PAPSTs) transfer PAPS from the cytosol into the Golgi lumen for the sulfation of sugar residues. Recently, two members of the PAPSTs, PAPST1 (SLC35B2) and PAPAT2 (SLC35B3), were identified in humans [3,4] and it was suggested that PAPSTs regulate the sulfation process in addition to sulfotransferases [5].

It has been reported that some sulfated structures alter the expression of molecules associated with the progression of cancer. In colon adenocarcinoma, the amount of sulfated glycoproteins decreased in the order normal mucosa, primary tumors, and metastatic tumors [6], and down-regulation of galactose-3-*O*-sulfotransferase-2 reduced the expression of sulfomucins in colon adenocarcinomas [7]. Instead, an increase in sialylation was associated with malignant transformation of colonic epithelial cells. The change of mucin production from sulfated to sialylated is a significant phenotypic alteration during tumor progression.

This study investigated the contribution of sulfation of PGs to radiation-induced apoptosis using PAPSTs and identified that the sulfation of keratan sulfate proteoglycan (KSPG) caused radioresistance in human Burkitt's lymphoma cell lines.

Abbreviations: CS, chondroitin sulfate; FGF, fibroblast growth factor; GAG, glycosaminoglycan; Gal, galactose; GlcNAc, *N*-acetylglucosamine; GlcNAc6ST, *N*-acetylglucosamine-6-*O*-sulfotransferase; HS, heparin sulfate; KS, keratan sulfate; MAPK, mitogen-activated protein kinase; PAPST, 3′-phosphoadenosine 5′-phosphosulfate transporter; PG, proteoglycan

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0014-5793/$36.00
http://dx.doi.org/10.1016/j.febslet.2012.12.002

2. Materials and methods

2.1. Cell culture and reagents

Human Burkitt's lymphoma cell lines Raji and Daudi were obtained from RIKEN Bio Resource Center and Namalwa was obtained from ATCC, and they were maintained in a medium consisting of RPMI 1640 medium (Gibco, Grand Island, NY, USA) supplemented with 10% heat-inactivated fetal calf serum (FCS). Anti-HS (HepSS-1, 10E4), anti-CS-A (2H6), anti-CS-D (MO-225), and anti-keratan sulfate (KS) (5-D-4, BCD-4) monoclonal antibodies were purchased from Siekagaku Kogyo (Tokyo, Japan).

2.2. Transfection of PAPST genes

Namalwa cells were transfected with pAMo vectors [8] containing human *PAPST1* or *PAPST2* genes by electroporation using a GenePulser apparatus (Bio-Rad, Richmond, CA, USA) (Namalwa-PAPST1, or Namalwa-PAPST2). The transfectants were selected by the addition of 0.6 mg/ml geneticin (G418) (Gibco) to the medium.

2.3. In vitro siRNA assay

Stealth RNAi is a type of chemically modified siRNA obtained from Invitrogen (Carlsbad, CA, USA). The synthesized oligonucleotides for the target site of each gene were as follows: PAPST1, 5′-ugg acc cag cua ugg uuc uuc cga u-3′ and 5′-auc gga aga acc aua gcu ggg ucc a-3′; the negative control for PAPST1, 5′-ugg acc ucg gua uug uuc cca cga u-3′ and 5′-auc gug gga aca aua ccg agg ucc a-3′; PAPST2, aac acu ucc uug ggc uac cug aau u-3′ and 5′-aau uca ggu agc cca agg aag ugu u-3′; the negative control for PAPST2, 5′-aac uuc cuu ggc gau ccg uac aau u-3′ and 5′-aau ugu acg gau cgc caa gga agu u-3′; CHST-2, 5′-ccu cuc gga aug aag gug uuc cgu a-3′ and 5′-uac gga aca ccu uca uuc cga gag g-3′; CHST-6, 5′-aua ggc auc aaa cac guc cau guc g-3′ and 5′-cga cau gga cgu guu uga ugc cua u-3′; and CHST-7, 5′-uaa aga guu cgc cca gga agg acg a-3′ and 5′-ucg ucc uuc cug ggc gaa cuc uuu a-3′. Each stealth RNAi duplex was transfected at a final concentration of 50 nM using Lipofectamine™ RNAiMAX in accordance with the manufacturer's protocol (Invitrogen).

2.4. Quantitative RT-PCR assay

A quantitative RT-PCR assay was performed to determine the amount of each transcript in Namalwa cells using a 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). The absolute amount of each transcript (copies/μl) was divided by that of β-actin (copies/μl) for normalization. Each normalized amount was further divided by that of the control sample to obtain the relative rate of expression.

2.5. Antibody array

The protein expression profile of Namalwa cells was determined using a Human Phospho-MAPK Array Kit in accordance with the manufacturer's protocol (R&D Systems, Minneapolis, MN, USA). Briefly, Namalwa-pAMo cells transfected with stealth RNAi targeting the *PAPST1* gene and Namalwa-PAPST1 cells transfected with the negative control stealth RNAi were irradiated with 20 Gy of X-rays 48 h after transfection. The cell lysates were prepared 6 h after irradiation and each array was incubated with 200–300 μg of lysate protein. The signals were generated using ECL Plus Western Blot Detection Reagents (GE Healthcare, Little Chalfont, UK) and detected using a luminescent image analyzer LAS-4000 mini (Fujifilm, Tokyo, Japan). The detected signals were quantified using Multi Gause ver 3.0 image analysis software (Fujifilm). Each value was calculated as a relative value compared with that in the positive control.

2.6. Western blot analysis

Phosphorylation of p38 and Akt was also examined by western blot analysis. A 50 μg sample of each lysate of transfectants was electrophoresed on an any KD Mini-PROTEAN TGX gel (BioRad) and transferred to an Immobilon PVDF membrane. Anti-phospho-p38 MAPK (Thr180/Tyr182) (9211) and anti-p38 MAPK (9212) antibodies (Cell Signaling Technology, Danvers, MA, USA) were used as probes at 1:500 dilution. An anti-β-Actin antibody (4967) was used as loading control at 1:1000 dilution (Cell Signaling Technology).

2.7. Apoptosis detection

Radiation-induced apoptosis was assessed by microscopic examination of nuclear morphology using Hoechst 33258 (Calbiochem, La Jolla, CA, USA), as described previously [9]. Briefly, cells were plated at a density of 3×10^5 cells per 3.5-cm dish and irradiated with X-rays. The condensed chromatin of nuclei was visualized by staining with 0.1 mg/ml of Hoechst 33258 after fixation with glutaraldehyde. The percentage of apoptotic cells was determined from the examination of 2,000 cells in 10 fields.

2.8. Irradiation

The cells were irradiated with X-rays using an X-ray generator Pantak HF-320S (Shimazu, Kyoto, Japan) at a dose rate of approximately 2.4 Gy/min.

3. Results

3.1. The overexpression of PAPST in Namalwa cells decreases radiation-induced apoptosis

We evaluated the transcript levels of PAPST1 and PAPST2 in a human B-lymphoma cell line, Namalwa cells (Fig. 1A). The level of PAPST1 transcripts was approximately 5-fold higher than that of PAPST2. Namalwa cells were transfected with an expression vector containing the coding sequence of human PAPST1 or PAPST2. The level of PAPST1 transcripts in Namalwa-PAPST1 cells was 2.5-fold higher than that in Namalwa-pAMo or Namalwa-PAPST2 cells. In contrast, the level of PAPST2 transcripts in Namalwa-PAPST2 cells was approximately 3-fold higher than that in Namalwa-pAMo or Namalwa-PAPST1 cells and reached 80% of the level of PAPST1 transcripts in Namalwa-pAMo cells.

The level of apoptosis was determined in each transfectant by Hoechst staining 24 h after irradiation (Fig. 1B). Irradiation increased the apoptosis of Namalwa-pAMo cells in a dose-dependent manner. In contrast, overexpression of PAPST decreased radiation-induced apoptosis in Namalwa cells. In particular, the most significant inhibition of apoptosis was seen at 20 Gy of irradiation, and the number of apoptotic cells in Namalwa-PAPST1 and -PAPST2 cells was approximately 60% of that in Namalwa-pAMo cells. Overexpression of PAPST1 and PAPST2 decreased apoptosis by almost the same extent.

3.2. Repression of PAPST increases radiation-induced apoptosis in Namalwa cells overexpressing each PAPST

Namalwa-PAPST1 or -PAPST2 cells were transfected with stealth RNAi targeted against the *PAPST1* or *PAPST2* gene, respec-