Cancer Letters 274 (2009) 201–207

Contents lists available at ScienceDirect

Cancer Letters

journal homepage: www.elsevier.com/locate/canlet

Depletion of nucleophosmin via transglutaminase 2 cross-linking increases drug resistance in cancer cells

Kang-Seo Park [a,b], Byeong-Gu Han [a,c], Kyoung Hwa Lee [a], Dae-Seok Kim [a,d], Jung-Mo Kim [a], Hyesung Jeon [e], Hyoun Sook Kim [f], Se Won Suh [f], Eunjoo H. Lee [g], Soo-Youl Kim [a], Byung Il Lee [a,*]

[a] Molecular Oncology Branch, Division of Basic and Applied Sciences, Research Institute, National Cancer Center, 111 Jungbalsan-ro, Ilsandong-gu, Gyeonggi 411-769, Republic of Korea
[b] Department of Biomedical Science, Graduate School, Kyung Hee University, Seoul 130-701, Republic of Korea
[c] Department of Biological Science, Sungkyunkwan University, Suwon 440-746, Republic of Korea
[d] Department of Molecular Cell Biology and Samsung Biomedical Research Institute, Sungkyunkwan University School of Medicine, Suwon 440-746, Republic of Korea
[e] Biomedical Research Center, Korea Institute of Science and Technology, Seoul 136-791, Republic of Korea
[f] Department of Chemistry, College of Natural Sciences, Seoul National University, Seoul 151-742, Republic of Korea
[g] Graduate School of East-West Medical Science, Kyung Hee University, Yongin 446-701, Republic of Korea

ARTICLE INFO

Article history:
Received 19 July 2008
Received in revised form 19 July 2008
Accepted 10 September 2008

Keywords:
Transglutaminase
Nucleophosmin
B23
Polymerization
Drug resistance

ABSTRACT

It has been suggested that nucleophosmin has an anti-apoptotic function via Bax binding. We found that nucleophosmin is a substrate of transglutaminase 2 (TGase 2) in cancer cells. Increased expression of TGase 2 expression is highly associated with drug resistance, and polymerization of nucleophosmin by TGase 2 also can be correlated with the drug resistance of cancer cells. In the present study, an accumulation of nucleophosmin in cytosol was detected when doxorubicin was treated to cancer cells, and it was found, moreover, that an increase of cytosolic nucleophosmin can result in drug-induced apoptosis. Nucleophosmin was polymerized by TGase 2, and the polymerization was inhibited with the TGase 2 inhibitor, cystamine, in vitro. The nucleophosmin level in the cytosolic cell fraction was reduced when TGase 2 was expressed, and the reduced nucleophosmin level was rescued by cystamine treatment. Moreover, nucleophosmin cross-linked by TGase 2 was eradicated in MCF7 cells via the ubiquitin-proteasomal pathway. In parallel with this nucleophosmin-level restoration, the pro-apoptotic Bax protein level was increased. Therefore, depletion of cytosolic nucleophosmin by TGase 2 can decrease Bax protein stability and lead to anti-apoptosis. Drug-resistant cancer cells became sensitive to doxorubicin treatment when nucleophosmin was expressed in cytosol. Taking these results together, it can be concluded that TGase 2 inhibits accumulation of cytosolic nucleophosmin through polymerization, which results in drug resistance in cancer cells.

1. Introduction

Transglutaminase 2 (TGase 2) catalyzes the formation of a covalent bond between the free amine groups in one protein and the protein-bound glutamines of another, creating cross-linked protein complexes [1]. TGase 2 is ubiquitously expressed, and is active in various physiological functions, such as blood clotting, wound healing, cell adhesion, barrier formation, and even apoptosis [2–6]. The increased expression of TGase 2 is associated with drug resistance in cancer [7–9], which is due to nuclear factor-κB (NF-κB) activation through the cross-linking and polymerization of free I-κB [10]. The drug resistance resulting from TGase 2 can be reversed by TGase 2 inhibition, and therefore, TGase 2 can be an attractive drug target in cases of chemo-resistant cancer [11].

* Corresponding author. Tel.: +82 31 920 2223; fax: +82 31 920 2006.
E-mail address: bilee@ncc.re.kr (B.I. Lee).
Abbreviation: TGase, transglutaminase.

0304-3835/$ - see front matter Crown Copyright © 2008 Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.canlet.2008.09.007

Human nucleophosmin, also known as B23, NO38, or numatrin, is an abundant multi-functional phosphoprotein in nucleoli [12–14]. Nucleophosmin also is active in many cellular functions, including ribosome biogenesis, histone assembly, regulation of DNA integrity, cell proliferation, and regulation of tumor suppressors [15–18]. This diversity of cellular activities reflects its function as either a potential oncogene or a potential tumor suppressor, depending on the circumstance [19]. Nucleophosmin is overexpressed in various tumors and has been proposed as a tumor marker. However, in some other human cancers, nucleophosmin is mutated, rearranged or deleted [19]. The N-terminal region of the nucleophosmin gene is translocated in lymphoid and myeloid disorders. This translocation produces of chimeric proteins, such as nucleophosmin-ALK, nucleophosmin-RARα, and nucleophosmin-MLF1 [20–22]. In recent reports, nucleophosmin is shown to be strongly correlated with apoptosis via the binding of Bax [23].

Recently, from the proteomic analysis of high-molecular-weight protein polymers in the cytosolic fraction of a doxorubicin-resistant breast cancer cell line, we found that nucleophosmin is a potential substrate of TGase 2, even though nucleophosmin exists predominantly in the nucleus [24]. In the present study, in order to elucidate the functional relationship between nucleophosmin and TGase 2, we tested whether polymerization of nucleophosmin by TGase 2 is associated with anti-apoptosis in a drug-resistant cancer cell line.

2. Materials and methods

2.1. Purification of nucleophosmin

The nucleophosmin B23 and B23.2 genes were subcloned into pET-21a(+) (Novagen) at the enzymes' NdeI/XhoI restriction sites. These vector constructions included an eight-residue tag (LEHHHHHH) to the C-terminus of the recombinant protein. The recombinant proteins were overexpressed in the *E. coli* strain Rosetta 2 (DE3) (Novagen). The cells were grown in Terrific Broth (MP Biomedicals) to an OD600 of 0.7 at 37 °C, and expression of the recombinant protein was induced by 0.6 mM isopropyl β-D-thiogalactopyranoside (IPTG) at 37 °C. The cell growth continued at 37 °C for 7 h after IPTG induction, and the cells were harvested by centrifugation. A cell pellet was resuspended in lysis buffer (25 mM Tris–HCl, pH 7.4, 138 mM NaCl, 2 mM KCl, 10% (v/v) glycerol, 1 mM phenylmethylsulfonyl fluoride, 10 μg of lysozyme) and maintained at −80 °C. The frozen cells were rapidly thawed and then homogenized by sonication. The crude lysate was centrifuged at 36000*g* for 1 h at 4 °C. The supernatant was applied to an Ni-NTA column (Qiagen), and the protein was eluted with elution buffer (500 mM imidazole, 500 mM NaCl, 20 mM Tris–HCl, pH 7.9, 10% (v/v) glycerol). The eluted protein was concentrated and applied to Superdex 200 prep grade (GE Healthcare) with a buffer of 100 mM Tris–HCl pH 7.5, 150 mM NaCl, 1 mM EDTA, 10 mM CaCl2, 5 mM DTT.

2.2. Polymerization of nucleophosmin

For the in vitro polymerization reactions, 40 μg of purified nucleophosmin was incubated with 1 milliunit of guineapig liver TGase 2 (Sigma) in a buffer of 100 mM Tris–HCl pH 7.5, 150 mM NaCl, 1 mM EDTA, 10 mM CaCl2, 5 mM DTT. The polymerization was analyzed by 4–12% NuPAGE gel electrophoresis (Invitrogen). For the electrophoresis, a 4× NuPAGE sampling buffer (Invitrogen) containing 9 M urea was added to the reaction mixture.

2.3. Transmission electron microscopy of nucleophosmin polymer

For transmission electron microscopy (TEM), 5 μl of protein solutions (250 μg/ml or 25 μg/ml nucleophosmin in Tris–HCl pH = 7.5, 100 mM NaCl) was adsorbed to a glow-discharged carbon-coated copper grid, washed with deionized water and stained with 2% uranyl acetate. The samples were imaged using a Tecnai F-20 electron microscope equipped with a field emission gun and operated at an acceleration voltage of 200 kV. Images were taken at magnifications of 50,000×, and 100,000×, respectively.

2.4. In vitro chaperone assay

The chaperone activity of nucleophosmin was measured using pocine heart citrate synthase (Sigma) as a substrate [25]. Citrate synthase (100 μg) and nucleophosmin (62.5 μg) were dissolved in 1 ml of assay buffer (100 mM Tris–HCl, pH 7.5, 150 mM NaCl, 10 mM CaCl2, 2 mM DTT, 1 mM EDTA). In order to polymerized nucleophosmin, 62.5 μg of nucleophosmin was incubated with 9 milliunits of guineapig liver TGase 2 for 13 h at 37 °C prior to the assay. The protein aggregation was monitored by measuring the absorbance at 360 nm in a spectrophotometer at 43 °C for 1 h.

2.5. Mass spectrometry

Matrix-assisted laser desorption ionization-mass spectrometry and a database search were performed to determine the important region for polymerization. SDS–PAGE gels containing proteins of interest were excised, destained with 50% acetonitrile in 0.1 M ammonium bicarbonate, and dried in a SpeedVac evaporator. The dried gel pieces were re-hydrated with 30 μl of 25 mM sodium bicarbonate, pH 8.8, containing 50 ng trypsin (Promega) at 37 °C overnight. α-Cyano 4 hydroxycinnamic acid (20 mg) (Bruker Daltonics, Bremen, Germany) was dissolved in 1 ml acetone:ethanol (1:2, v/v), and 0.5 μl of the matrix solution was mixed with an equivalent volume of sample. An analysis was performed using an Ultraflex TOF/TOF system (Bruker Daltonics) at Proteomics Core, National Cancer Center, Korea. The Ultraflex TOF/TOF system was operated in positive ion reflect mode. Each spectrum was the cumulative average of 250–450 laser shots. The mass spectra were first calibrated in the closed external mode using the peptide calibration standard II (Bruker Daltonics), sometimes using the internal statistical mode to achieve maximum calibration mass accuracy, and analyzed with FlexAnalysis software, version 2.4 (Bruker Daltonics). The peptide mass peaks from each spectrum were submitted to the Mascot peptide mass fingerprinting search form (http://www.matrixscience.com) for analysis