Available online at www.sciencedirect.com
ScienceDirect
Life Sciences 80 (2007) 2051–2059
Life Sciences
www.elsevier.com/locate/lifescie

Involvement of nPKC-MAPK pathway in the decrease of nucleophosmin/B23 during megakaryocytic differentiation of human myelogenous leukemia K562 cells

Chih-Chung Chou [a], Benjamin Yat-Ming Yung [b], Chen-Ya Hsu [a,*]

[a] *Department and Graduate School of Biotechnology, Fooyin University, 151 Chin-Hsueh, Rd., Ta-Liao Hsiang, Kaohsiung Hsien, 831 Taiwan, ROC*
[b] *Genomics Research Center and Cancer Biochemistry Laboratory, Department of Pharmacology, College of Medicine, Chang Guang University, 259 Wen-Hwa 1st Rd, Kwei-San Hsiang, Tao-Yuan Hsien, 333, Taiwan, ROC*

Received 26 September 2006; accepted 7 March 2007

Abstract

Human myelogenous leukemia K562 cells were induced to undergo megakaryocytic differentiation by long-term treatment with phorbol ester 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA). The protein level of nucleophosmin/B23 (NPM/B23), a nucleolar protein, was substantially decreased upon TPA treatment. In this study, we found that the proteasome inhibitors blocked the decrease of NPM/B23 protein in response to TPA, suggesting the proteasomes were involved in the downregulation of NPM/B23 upon megakaryocytic differentiation. To investigate the signaling pathway in the downregulation of NPM/B23 during early TPA-induced megakaryocytic differentiation of K562 cells, K562 cells were treated with TPA in the presence of the PKC isozyme-selective inhibitors, GF109203X and Gö 6976, or MEK1 inhibitor, PD98059. The decrease of NPM/B23 protein in the TPA-treated K562 cells was blocked by GF109203X but not by Gö 6976, suggesting the involvement of novel PKCs in the downregulation of NPM/B23 during TPA-induced megakaryocytic differentiation of K562 cells. The application of MEK1 inhibitor PD98059 upon TPA treatment blocked the TPA-induced decrease of NPM/B23 protein and aborted the megakaryocytic differentiation but not to break through the cell growth arrest. Unlike NPM/B23, the degradation of nucleolin in the TPA-treated K562 cells could not be blocked by PD98059 while the TPA-induced megakaryocytic differentiation was abrogated. The decrease of NPM/B23 protein seems to be more correlated with the novel PKC-MAPK-induced megakaryocytic differentiation than another nucleolar protein, nucleolin. Taken together, our results indicated that novel PKC-MAPK pathway was required for the decrease of NPM/B23 during TPA-induced megakaryocytic differentiation.

Keywords: Nucleophosmin/B23; Nucleolin; Megakaryocytic differentiation

Introduction

One important difference between cancer and normal cells is the hyperactivity and the pleomorphism of nucleoli in cancer cells (Bush et al., 1963). Nucleophosmin/B23 (NPM/B23), a nucleolar protein, is significantly more abundant in cancer cells than in normal resting cells (Chan et al., 1989). Overexpression of NPM/B23 in NIH 3T3 cells results in malignant transformation; thus, NPM/B23 seems to be associated with oncogenic activity (Kondo et al., 1997). NPM/B23 is downregulated in HL-60 cells during retinoic acid-induced differentiation (Hsu and Yung, 1998) and sodium butyrate-induced apoptosis (Liu and Yung, 1998). Blockage of NPM/B23 expression with its antisense oligonucleotides significantly potentiates the induction of differentiation and apoptosis (Hsu and Yung, 1998; Liu and Yung, 1998). Overexpression of NPM/B23 in HL-60 cells or K562 cells can lead to resistance to RA- or TPA-induced differentiation (Hsu and Yung, 2000, 2003). These studies indicate that NPM/B23 plays a role in the regulation of nucleolar function for cellular growth, differentiation, and apoptosis. However, little is known regarding the signaling pathway in the downregulation of NPM/B23 during hematopoietic cell differentiation. Nucleolin is another abundant nucleolar protein that is believed to be a pre-ribosomal RNA chaperone and may interact with NPM/B23 in vivo (Liu and

* Corresponding author. Tel.: +886 7 7811151x645; fax:+886 7 7862707.
E-mail address: fydr004@yahoo.com.tw (C.-Y. Hsu).

0024-3205/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.lfs.2007.03.004

Yung, 1999). Nucleolin and NPM/B23 are major nucleolar proteins of exponentially growing eukaryotic cells (Derenzini, 2000). Previous studies have shown that nucleolin fluctuates in parallel to DNA synthesis (Derenzini et al., 1995). Increased stability of nucleolin in actively dividing cells is presumably by inhibition of its self-cleaving activity (Chen et al., 1991). Unlike nucleolin, the downregulation of NPM/B23 is not proliferation-dependent but apoptosis-dependent (Chou and Yung, 2001).

The K562 cell line was originally established from the pleural effusion of a patient with chronic myelogenous leukemia (CML) in the blastic phase (Tabilio et al., 1983). The K562 cell line can be induced to differentiate towards megakaryocytic differentiation by the phorbol ester 12-*O*-tetradecanoylphorbol-13-acetate (TPA) and resulted in loss of proliferative capacity (Alitalo, 1990). Previous studies have shown that activation of protein kinase C (PKC) by TPA induces megakaryocytic differentiation of K562 cells (Alitalo, 1990; Long et al., 1990). A role for PKC signaling in megakaryocytic differentiation has been established by numerous experiments. Recent studies using primary human progenitors confirmed the promegakaryocytic effects of phorbol ester and showed such effects to be inhibitable by the PKC antagonists GF109203X and RO318220 (Lumelsky and Schwartz, 1997). The PKC serine/threonine kinase family consists of at least 12 distinct isozymes based on their biochemical properties and sequence homologies. They have been divided into three groups: the classical PKCs (cPKC-α,-βI,-βII and -γ) which are activated in a diacylglycerol- and calcium-dependent manner; the calcium-independent but diacylglycerol-dependent novel PKCs (nPKC-δ,-ε,-η,-θ and -μ); and a third group consisting of atypical PKCs (aPKC-ζ,-ι and -λ). The members of this last group of isozymes are unresponsive to diacylglycerol and calcium and, in contrast to c- and nPKCs, do not respond to phorbol esters (Nishizuka, 1992; Hug and Sarre, 1993; Dekker and Parker, 1994). Signal transduction of PKC may influence megakaryocytic differentiation through several isozymes. Multiple PKC isozymes, in particular α, βI, and δ, possess the capacity to activate the Raf-MEK-ERK pathway (Schonwasser et al., 1998; Ueda et al., 1996). In K562 cells, PKC-mediated, sustained activation of the Raf-MEK-ERK signaling pathway is necessary for initiation of megakaryocytic differentiation (Racke et al., 1997).

To examine the contribution of specific PKC isozymes on decrease of NPM/B23 protein during TPA-induced megakaryocytic differentiation of K562 cells, we employed isozyme-selective pharmacologic agents, GF109203X and Gö 6976. GF109203X inhibits both cPKC and nPKC isozymes (Toullec et al., 1991). Gö 6976 is an inhibitor only of cPKCs but not of nPKCs (Martiny-Baron et al., 1993). In the present study, we reported that the decrease of NPM/B23 and nucleolin protein in the TPA-treated K562 cells was blocked by GF109203X but not blocked by Gö 6976. The results suggested the involvement of nPKCs in the downregulation of NPM/B23 and nucleolin during TPA-induced megakaryocytic differentiation of K562 cells. We also addressed whether ERK/MAPK kinase pathway influenced the decrease of NPM/B23 and nucleolin protein during TPA-induced megakaryocytic differentiation of K562 cells. We found that the decrease of NPM/B23 protein in the TPA-treated K562 cells was blocked by MEK1 inhibitor, PD98059. However, the degradation of nucleolin in the TPA-treated K562 cells could not be blocked by PD98059 while the TPA-induced megakaryocytic differentiation was abrogated. These results suggested the involvement of nPKC and ERK/MAP kinase pathway in the downregulation of NPM/B23 protein during TPA-induced megakaryocytic differentiation of K562 cells. The decrease of NPM/B23 protein seems to be more correlated with the TPA-induced megakaryocytic differentiation than nucleolin. The ubiquitin–proteasome pathway, the major nonlysosomal degradation system, has been implicated in regulating the levels of many cellular proteins (Goldberg et al., 1997). Thus proteasomes are thought to be an important soluble proteolytic complex responsible for various biological events (Shimbara et al., 1992; Spataro et al., 1998). In the present study, we reported that the decrease of NPM/B23 and nucleolin protein in the TPA-induced megakaryocytic differentiation was inhibited by the proteasome inhibitors, suggesting that the proteasomes were involved in the TPA-induced downregulation of NPM/B23 and nucleolin.

Materials and methods

Drugs and antibodies

All chemicals and anti-β-actin monoclonal antibody (mAb) were purchased from the Sigma Chemical Co. 12-*O*-tetradecanoylphorbol-13-acetate (TPA) and cycloheximide was dissolved in ethanol. MG132, ALLN, NLVS, lactacystin, GF109203X, Gö 6976 and PD98059 were dissolved in dimethyl sulfoxide. Anti-NPM/B23 mAb was kindly provided by Dr. P. K. Chan (Department of Pharmacology, Baylor College of Medicine, Houston, TX). Mab to nucleolin was kindly provided by Dr. N. H. Yeh (Institute of Microbiology and Immunology, National Yang Ming University, Taipei, Taiwan). The monoclonal anti-phospho-ERK Ab and anti-ERK Ab were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The monoclonal anti-CD41a-FITC Ab and anti-CD61-FITC Ab were purchased from BD Pharmingen (San Diego, CA).

Cell culture

The K562 leukemia cells were grown in RPMI-1640 medium (Invitrogen) supplemented with 10% heat-inactivated fetal bovine serum (Hyclone, UT), 2 mM glutamine, 50 units/ml penicillin and 50 μg/ml streptomycin in a 5% CO_2 humidified incubator at 37 °C. Every 24 h for 3 days, cultures were harvested and monitored for cell number by counting cell suspensions with hemocytometer. Cell viability was assessed by exclusion of 0.2% trypan blue.

Assays of cellular differentiation

TPA-induced megakaryocytic differentiation of K562 cells was assayed by measuring the expression of differentiation-specific membrane antigens (CD41a and CD61) (Gewirtz et al.,