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Proteins implicated in the increase of adhesivity induced by suberoylanilide hydroxamic acid in leukemic cells

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ABSTRACT

We have previously shown that suberoylanilide hydroxamic acid (SAHA) treatment increases the adhesivity of leukemic cells to fibronectin at clinically relevant concentrations. Now, we present the results of the proteomic analysis of SAHA effects on leukemic cell lines using 2-DE and ProteomLab PF2D system. Histone acetylation at all studied acetylation sites reached the maximal level after 5 to 10 h of SAHA treatment. No difference in histone acetylation between subtoxic and toxic SAHA doses was observed. SAHA treatment induced cofilin phosphorylation at Ser3, an increase in vimentin and paxillin expression and a decrease in stathmin expression as confirmed by western-blotting and immunofluorescence microscopy. The interaction of cofilin with 14-3-3 epsilon was documented using both Duolink system and coimmunoprecipitation. However, this interaction was independent of cofilin Ser3 phosphorylation and the amount of 14-3-3-ε-bound cofilin did not rise following SAHA treatment. SAHA-induced increase in the cell adhesivity was associated with an increase in PAK phosphorylation in CML-T1 cells and was abrogated by simultaneous treatment with IPA-3, a PAK inhibitor. The effects of SAHA on JURL-MK1 cells were similar to those of other histone deacetylase inhibitors, tubastatin A and sodium butyrate. The proteome analysis also revealed several potential non-histone targets of histone deacetylases.

1. Introduction

Histone deacetylases (HDACs) are enzymes that remove acetyl groups from lysines of various proteins. The most prominent HDAC targets are histones, which are tightly bound to nuclear DNA and are important for its organization into nucleosomes. Different posttranslational modifications, including acetylation of core histones, regulate the rate of gene transcription, thus HDAC inhibition results in changes in the expression level of a large number of genes [1]. HDACs target not only the histones, but also many other protein substrates, e.g. the heat shock protein HSP90 or tubulin [2]. HDAC inhibitors (HDACi) are emerging as promising drugs in treatment of cancer and as anti-inflammatory agents [3,4]. HDACi induce different and pleiotropic effects in various transformed cells including growth arrest, activation of the extrinsic or intrinsic apoptotic pathways, autophagy, reactive oxygen species-induced cell death, mitotic cell death and senescence [5]. On the other hand, normal cells are considerably more resistant to HDACi. Suberoylanilide hydroxamic acid (SAHA) was the first HDACi approved by U.S. Food and Drug

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Administration for treatment of cutaneous T-cell lymphoma [6]. In clinical trials, SAHA shows activity against hematologic and solid cancers at doses well tolerated by patients. In leukemia cells, the main mechanism of toxic SAHA action may be an accumulation of excessive DNA damage leading to activation of DNA repair response and apoptosis [7]. A set of new potential non-HDAC targets of SAHA has recently been described, too [8].

So far, there have been only a few attempts to identify SAHA-induced changes in cell proteomes. Proteomic analysis of SAHA effects was performed in cell lines derived from liver cancer [9], cervical cancer [10] and in the plasma of dystrophic mice [11]. The analysis of the leukemic cell line Jurkat revealed changes in proteins implicated in reactive oxygen species production and autophagy [12].

We have previously shown that SAHA can induce an increase in leukemic cell binding to fibronectin [13]. We described this effect in human cell lines originating from chronic myelogenous leukemia (CML), acute myeloid leukemia, and T-cell lymphoma. Due to its broad-range anti-proliferative and pro-apoptotic effects, SAHA is now being considered for use as a single agent or in combination with more specific anti-cancer drugs; e.g. synergy of SAHA action with tyrosine kinase inhibitors used in CML treatment (imatinib mesylate and dasatinib) has been documented [14,15]. Also, inhibition of sirtuin1, which belongs to a specific HDAC class (not inhibited by SAHA), was recently reported to potentiate imatinib effects on CML leukemia stem cells [16]. However, these experiments were performed only *ex vivo* and thus could not show possible effect these drugs could have on cell adhesion to the bone marrow which is known to provide protection against imatinib cytotoxicity [17–19]. The expression of the fusion protein Bcr–Abl, which is characteristic for CML, not only confers growth advantage and resistance to the apoptosis, but also alters cell adhesion to the bone marrow stroma and the ability to migrate towards growth factors such as SDF-1 [20,21]. Restored adhesivity of CML cells to the bone marrow may increase the number of quiescent leukemic stem cells and aggravate the residual disease. In this work, we present the results of the proteomic analysis of SAHA-induced changes in the CML cell line JURL-MK1, with the emphasis on the proteins which could be implicated in the observed changes in cellular adhesivity.

Cell interaction with extracellular matrix proteins is mediated prevalently by integrin receptors and comprises formation of large multiprotein structures at integrin cytoplasmic parts. These structures, which are called focal adhesions in adherent cell types, involve adaptor proteins (e.g. paxillin) and signaling molecules (e.g. FAK kinase, Src family kinases) among others [22]. The intracellular signaling regulates the stability and turnover rate of the adhesion structures and promotes either firm attachment to the extracellular matrix or cell migration [23]. In mature leukocytes, the adhesive structures are called podosomes [24]. While the molecular composition of podosomes is similar to that of focal adhesions, podosomes are characteristic by different morphology, faster turnover and by the presence of matrix-degrading proteins. The precise nature of adhesive structures in hematopoietic precursor cells and in leukemic blasts is not known but their composition and regulation very likely resemble that of focal adhesions and podosomes.

2. Material and methods

2.1. Chemicals

Fibronectin fragment (120 kDa cell attachment region) was purchased from Chemicon International (CA, U.S.A.). Suberoylanilide hydroxamic acid (SAHA) was obtained from Cayman Chemical (distributed by the Axxora platform) and 2 mM stock solution was prepared in dimethyl sulfoxide (DMSO). Tubastatin A was supplied by BioVision (CA, U.S.A.) and 20 mM stock solution was made in DMSO. Further dilution of both effectors was made in sterile water. Sodium butyrate, trichostatin A , protease inhibitor cocktail (cat. no. P8340) and phosphatase inhibitor cocktail 2 (cat. no. P 5726) were purchased from Sigma (Prague, Czech Republic). IPA-3 was purchased from Santa Cruz and 20 mM stock solution was made in DMSO.

The source and the identification of primary non-conjugated antibodies were as follows: anti-vimentin (Sigma-Aldrich), clone V9, cat. no. V6389; anti-phospho-cofilin (pSer3) (Sigma), cat. no. C8992; anti-paxillin (BD Transduction Laboratories™), cat. no. 610051; anti-acetylated α tubulin 6-11B-1 (Abcam), cat. no. ab24610; anti-stathmin 1 (Abcam), cat. no. ab11269; anti-phospho-stathmin (Ser38) (Cell Signaling), cat. no. #3426; anti-phospho-src family (Tyr416) (Cell Signaling Technology®), cat. no. #2101; anti-14-3-3 (Chemicon® International), clone 8C3, cat. no. MAB3053; anti-14-3-3 epsilon T-16 (Santa Cruz), cat. no. sc-1020; anti-eIF5A [EP527Y] (Abcam), cat. no. ab32407; anti-cofilin FL 166 (Santa Cruz), cat. no. sc-33779; anti-β actin (Sigma), clone AC-15, cat. no. A 5441; anti-histone acetylation-specific and C-terminal (Abcam); anti-HSC70 [N27F34] (Abcam), cat. no. ab90347; anti-SFPQ (ProteinTech Group, Inc), cat. no. 15585-1-AP; anti-phospho-PAK1,2 (Cell Signaling), cat. no. 2601; anti-PAK1 (Cell Signaling), cat. no. 2602. The anti-α-tubulin antibody conjugated with AlexaFluor 488®, clone/PAD B-5-1-2, cat. no. 32–2588 was from Invitrogen. Goat anti-mouse and anti-rabbit secondary antibodies for ECL were from Thermo Scientific (cat. nos 31430, 31460), AlexaFluor 488-conjugated secondary antibodies from Invitrogen. Phycoerythrin-conjugated anti-rabbit secondary antibody was from Vector Laboratories, CA.

2.2. Cell culture

JURL-MK1 and CML-T1 cells (both derived from CML) were purchased from DSMZ (German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany). K562 (another CML cell line) and HL-60 (acute myelogenous leukemia) cells were from the European Collection of Animal Cell Cultures (Salisbury, UK). The cells were cultured in RPMI 1640 medium supplemented with 10% fetal calf serum, 4 mM L-alanyl-L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin at 37 °C in 5% CO_2 humidified atmosphere. To determine the cell density and the dead cell fraction, the cells were stained with Trypan blue and counted using the automated cell counter TC10 (BioRad).

2.3. Measurement of cell adhesivity to FN

The method for assessment of cellular adhesivity to FN has been described previously [13]. Briefly, the cells (1×10^4) were