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Short communication

ERK1/2 activation plays important roles in the opposite effects of Trichostatin A in non-cancer and cancer cells

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ABSTRACT

Histone deacetylase (HDAC) inhibitors are candidates of anti-cancer drugs as they can effectively kill cancer cells while have little or no toxicity to non-cancer cells, but the molecular mechanism underlying this process remains unclear. We previously reported that HDAC inhibitors could protect normal mouse hepatocytes from apoptosis induced by transforming growth factor-β1 (TGF-β1) with the requirement of extracellular signal-regulated kinase 1/2 (ERK1/2). In this study, we investigate the roles of trichostatin A (TSA), a typical HDAC inhibitor, on three non-cancer cell lines AML-12, MDCK and 3T3-L1, and four cancer cell lines Hep-3B, HeLa, A549 and MCF-7. TSA is a fermentation product of *Streptomyces* originally used as an antifungal agent. Our results showed that TSA blocked not only the TGF-β1-induced apoptosis but also serum starvation-induced apoptosis in all the non-cancer cells, whereas it could induce strong apoptosis in all the cancer cells. Further investigation revealed that TSA can induce the activation of ERK1/2 in the three non-cancer cells but not in the cancer cells. In summary, these findings indicated that TSA protect non-cancer cells from apoptosis *via* activating ERK1/2, providing a useful insight into the better application of HDAC inhibitors in cancer therapy.

1. Introduction

Apoptosis is a highly controlled process that has been implicated in various physiological and pathological events (Sánchez-Capelo, 2005). During apoptosis, inflammatory response does not happen, so inducing apoptosis to kill tumor is an effective method in cancer therapy (Rowinsky, 2005). Lots of drugs can induce significant apoptosis in cancer cells, but most of them also have strong side effects to the surrounding non-cancer cells (Dean et al., 2005; Rowinsky, 2005). Therefore, the research and development of anti-cancer drugs with high-quality and tumor-selectivity is necessary for cancer treatment.

Abbreviations: HDAC, histone deacetylase; MAPK, mitogen-activated protein kinase; ERK1/2, extracellular signal-regulated kinase 1/2; TSA, trichostatin A; MEK1, MAPKK1, mitogen-activated protein kinase kinase 1; TGF-β, transforming growth factor-β; p-ERK1/2, phospho-ERK1/2; MDCK, Madin-Darby canine kidney; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; FACS, Flow cytometry analysis; PI, propidium iodine; VPA, valproic acid.

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Histone deacetylases (HDACs) regulate various biological events, including embryonic development, stress response, cytoskeletal dynamics, the integrity of endothelium, cell apoptosis and survival, and epithelial–mesenchymal transition (Haberland et al., 2009; Lei et al., 2010a, 2010b). The compounds that contain hydrophobic tails and the Zn^{2+} chelator hydroxyamic acid as a head group, are potent inhibitors of HDACs and are powerful tools to study the roles of HDACs (Singh et al., 2005; Vannini et al., 2004). It was recently reported that HDAC inhibitors can induce strong apoptosis in cancer cells, and also suppress tumor growth and progression, induce differentiation of transformed cells and inhibit tumor angiogenesis and invasion *in vivo* (Bolden et al., 2006; Minucci and Pelicci, 2006; Wilson, 2006). In clinical trials, HDAC inhibitors had pronounced antitumor activity with promising clinical results (Bolden et al., 2006; Ramalingam et al., 2010). Hence, HDAC inhibitors have been considered as potential drugs for cancer treatment. Till now, some of the underlying mechanisms by which HDAC inhibitors sensitize cancer cells to apoptosis have not been uncovered. In addition, it was reported that HDAC inhibitors have strong tumor cell selectivity as they are less or non-toxic to normal cells (Marks, 2007; Minucci and Pelicci, 2006). However, the reason why HDAC inhibitors have little toxicity to non-cancer cells has not been explored.

Extracellular signal-regulated kinase 1/2 (ERK1/2) is one kind of the most important member of Mitogen-activated protein kinases (MAPKs), which respond sensitively to diverse extracellular stimuli in mediating cell proliferation, differentiation, migration, stress responses, inflammation, and apoptosis (Marshall, 1995; Owens and Keyse, 2007). ERK interacts with more than 170 proteins including many substrates (Roberts and Der, 2007; von Kriegsheim et al., 2009). Thus, the interaction of ERK with different proteins in response to distinct stimuli may influence substrate specificity and lead to precise biological outcomes. The ERK1/2 pathway has long been associated with cell proliferation and survival (Ballif and Blenis, 2001; Junttila et al., 2008). Recently, it has been shown that ERK1/2 can inactivate the proapoptotic protein BAD and up-regulate anti-apoptotic Bcl-2, Bcl-xL, and Bcl-1 proteins (Junttila et al., 2008). Moreover, ERK1/2 activity can suppress Fas-mediated apoptosis by inhibiting the formation of the death-inducing signaling complex (Geryk-Hall et al., 2010; Junttila et al., 2008).

Our pervious study has demonstrated that three structural-unrelated HDAC inhibitors, including trichostatin A (TSA), sodium butyrate (NaBu) and MS-275, can activate ERK1/2 to suppress transforming growth factor-β1 (TGF-β1)-induced apoptosis in hepatocytes (Lei et al., 2010b). However, whether regulation of ERK1/2 is a common mechanism of HDAC inhibitors to block/induce apoptosis in non-cancer/cancer cells remains unclear. In this study, we found that TSA suppressed apoptosis induced by TGF-β1 or serum starvation in three non-cancer cell lines, but it induced strong apoptosis in four cancer cell lines. Next, the examination of ERK1/2 phosphorylation level in all the cells after TSA treatment revealed that ERK1/2 plays important roles in the different effects of TSA in non-cancer and cancer cells.

2. Materials and methods

2.1. Materials

The normal murine hepatocyte AML-12, murine adipocyte 3T3-L1, canine kidney cell line MDCK, human hepatocellular carcinoma cell line Hep-3B, non-small lung cancer cell line A549, cervical carcinoma cell line HeLa and mammary carcinoma cell line MCF-7 were purchased from American Type Culture Collection (Manassas, VA, USA). Human recombinant TGF-β1 was from Chemicon (Rosemont, IL, USA). Antibodies for phospho-ERK1/2 (p-ERK1/2), ERK2 and HRP-conjugated secondary antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA, USA). All other reagents were from Sigma (St. Louis, MO, USA) unless otherwise indicated. The protein concentration was determined by a protein assay kit with bovine serum albumin (BSA) as a standard (Bio-Rad, Hercules, CA, USA).

2.2. Cell culture

Cell culture reagents were from Invitrogen (Carlsbad, CA, USA). AML-12 murine hepatocytes were cultured in a 1:1 mixture of Dulbecco's modified Eagle's medium (DMEM) and Ham's F12 containing 10% fetal bovine serum (FBS) and supplied with insulin (5 μg/ml), transferrin (5 μg/ml), selenium (5 μg/ml), dexamethasone (40 ng/ml), penicillin (100 units/ml), and streptomycin (100 μg/ml). MDCK, A549, HeLa, MCF-7 and 3T3-L1 cells were cultured in DMEM containing 10% FBS and supplied with penicillin (100 units/ml) and streptomycin (100 μg/ml), and Hep-3B cells were cultured in modified Eagle's medium (MEM) containing 10% FBS and supplied with penicillin (100 units/ml) and streptomycin (100 μg/ml). The cells were incubated at 37 °C in a humidified atmosphere with 5% CO_2. Experiments were performed when cells reached 40–60% confluence.

2.3. Flow cytometry analysis (FACS) of apoptosis

FACS assay was performed mainly as reported previously (Lei et al., 2010b). After indicated treatment, cells were trypsinized and fixed with 70% ethanol at 4 °C for over 2 h. The cells were then pelleted and washed with PBS plus 20 mM EDTA. RNA was removed by incubating samples with RNase A (1 mg/ml) at 37 °C for at least 1 h. Cells were then stained with propidium iodine (PI, final concentration: 30 μg/ml). PI-positive cells were detected by flow cytometry analysis (Becton Dickinson FACS Calibur, BD Biosciences, San Jose, CA, USA). Cell apoptotic rate was measured by the percentage of sub-G1 DNA amount.

2.4. Preparation of cell lysate and immunoblotting

Cells were lysed in lysis buffer containing 50 mM HEPES (pH 7.4), 5 mM EDTA, 50 mM NaCl, 1% Triton X-100, 50 mM NaF, 10 mM $Na_4P_2O_7 \cdot 10H_2O$, 5 μg/ml aprotinin, 5 μg/ml leupeptin, 1 mM Na_3VO_4, and 1 mM phenylmethylsulfonyl fluoride at 4 °C. The lysate was clarified by centrifugation (12,000 g, 15 min, 4 °C). The sample (containing 30 μg proteins) was loaded on an SDS-polyacrylamide gel and