Letter to the Editor.

Inhibition of MEK/ERK signaling synergistically potentiates histone deacetylase inhibitor-induced growth arrest, apoptosis, and acetylation of histone H3 on p21*waf1* promoter in acute myelogenous leukemia cell

Histone deacetylase inhibitors (HDACIs) have emerged as a potentially promising new class of anticancer drugs.[1] These include the hydroxamic acid-derived suberoylanilide hydroxamic acid (SAHA, Vorinostat), LBH 589, tricostatin A (TSA), cyclic depsipeptide FR901228, and the benzamide MS-275.[5] HDACIs induce growth arrest and apoptosis of cancer cells by manipulating the transcription of genes involved in regulation of the cell cycle and apoptosis, as well as differentiation.[1] For example, we previously showed that SAHA induces growth arrest and apoptosis of human mantle cell lymphoma cells in association with induction of histone acetylation of the p21*waf1* promoter region, resulting in upregulation of the p21*waf1* protein.[2]

This study found that MS-275 induces growth arrest of human acute myelogenous leukemia (AML) HL-60 and NB4 cells, as well as freshly isolated leukemia cells from individuals with AML with IC50s values less than 1 μM on day 2 of culture, as measured by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay and thymidine uptake, respectively (Fig 1a, Table 1). Western blot analyses showed that exposure of these cells to MS-275 downregulated levels of antiapoptotic molecules Bcl-2 and Mcl-1, and markedly increased levels of p21*waf1* in conjunction with induction of acetylation of histone H3 proteins (Fig 1b). MS-275 also downregulated levels of p-ERK in these cells (Fig 1b). We next explored the drug interaction of MS-275 and AZD6244 (ARRY-142886),[3] the specific inhibitor of MEK/ERK activity, in AML cells. Interestingly, AZD6244 synergistically potentiated MS-275-induced growth arrest and

apoptosis of these cells in association with enhanced induction of p21^{waf1} (Supplementary Figs 1a-e). We further explored the molecular mechanisms by which blockade of MEK/ERK signaling enhanced MS-275-induced expression of p21^{waf1}; AZD6244 increased MS-275-induced acetylation of histone H3 proteins in AML cells (Fig 2a). Of note, chromatin immunoprecipitation assay found that combination of AZD6244 and MS-275 synergistically induced acetylation of histone H3 on the *p21*^{waf1} promoter in AML cells (Fig 2b). Moreover, MS-275 or AZD6244 either alone modestly downregulated levels of p-histone H3 and combination of both at the same concentration further downregulated levels of p-histone H3 in these cells (Supplementary Fig 2). This is the first observation that blockade of MEK/ERK signaling modulates the epigenetic action of HDACI. Phosphorylation of histone H3 coincided with chromatin condensation in mitosis in eukaryotic cells.[4,5] Inhibition of p-histone H3 by this MEK inhibitor may cause chromatin relaxation, leading to susceptibility to HDACI-induced acetylation of the gene promoter.

A recent phase I clinical trial has evaluated efficacy and safety of MS-275 in 38 individuals with relapsed or refractory AML. MS-275 induced acetylation of histone H3/H4, expression of p21^{waf1} and activation of caspase 3 in bone marrow mononuclear cells, although no responses were observed by classical criteria.[6]

Taken together, combination of MS-275 and AD6244 may be useful for treatment of individuals with AML. Further studies are required to verify the molecular mechanisms by which blockade of MEK/ERK signaling affects chromatin remodeling.

Supplementary information accompanies the paper on the Leukemia website (http://www.nature.com/leu).

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Figure Legends

Fig 1. (a), Antiproliferative effects of MS-275 against leukemia cells. The human leukemia HL60 and NB4 cells were plated in 96-well plates and cultured with various concentrations of MS-275 (0.1-6 μM). After 2 days, cell proliferation was measured by MTT assay. Results represent the mean ± SD of three experiments performed in triplicate. **(b), MS-275 modulates levels of antiapoptotic and cell cycle related proteins and induces acetylation of histone H3. Western blot analysis.** HL60 and NB4 cells were cultured with various concentrations of MS-275 (0.25-1 μM). After 48 hours, cells were harvested and subjected to Western blot analysis. The membranes were sequentially probed with the indicated antibodies. The figures represent one of the three experiments performed independently. Ac-histone H3, acetylated-histone H3.

Fig 2. AZD6244 enhanced MS-275-induced acetylation of histone H3. (a) Western blot analysis. HL60 and NB4 cells were cultured in the presence of either MS-275 (0.5 μM) and/or AZD6244 (0.25 μM). After 6 hours, cells were harvested and nuclear proteins were prepared and subjected to Western blot analysis. The membranes were sequentially probed with anti-acetyl-histone H3 and anti-α-tubulin antibodies. The figure is representative of three similar experiments. Ac-histone H3, acetylated-histone H3. **(b) Chromatin immunoprecipitation assay.** Acetylation of histone H3 in the *p21^{waf1}* promoter was analyzed by chromatin immunoprecipitation assay. HL60 and NB4 cells were cultured in the presence of either MS-275 (0.5 μM) and/or AZD6244 (0.25 μM). After 6 hours, cells were harvested and subjected to chromatin immunoprecipitation assay. Anti-acetylated histone H3 antibody or rabbit IgG was used

to immunoprecipitate soluble chromatin from these cells. The recovered DNAs were subjected to PCR using primers for *p21*^{waf1}promoter. The band intensity was measured by densitometry. The result is representative of two additional experiments performed independently.

Figure 1 a

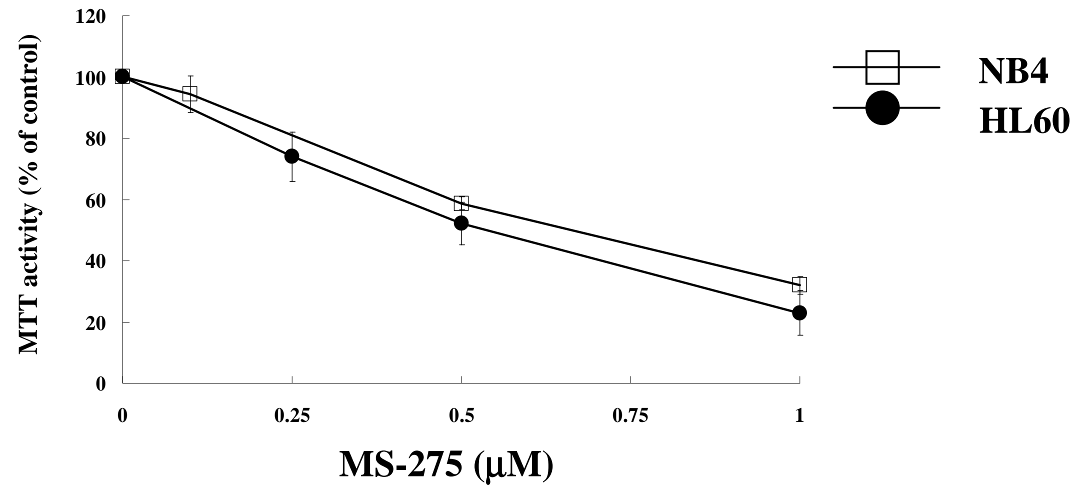

Figure 1

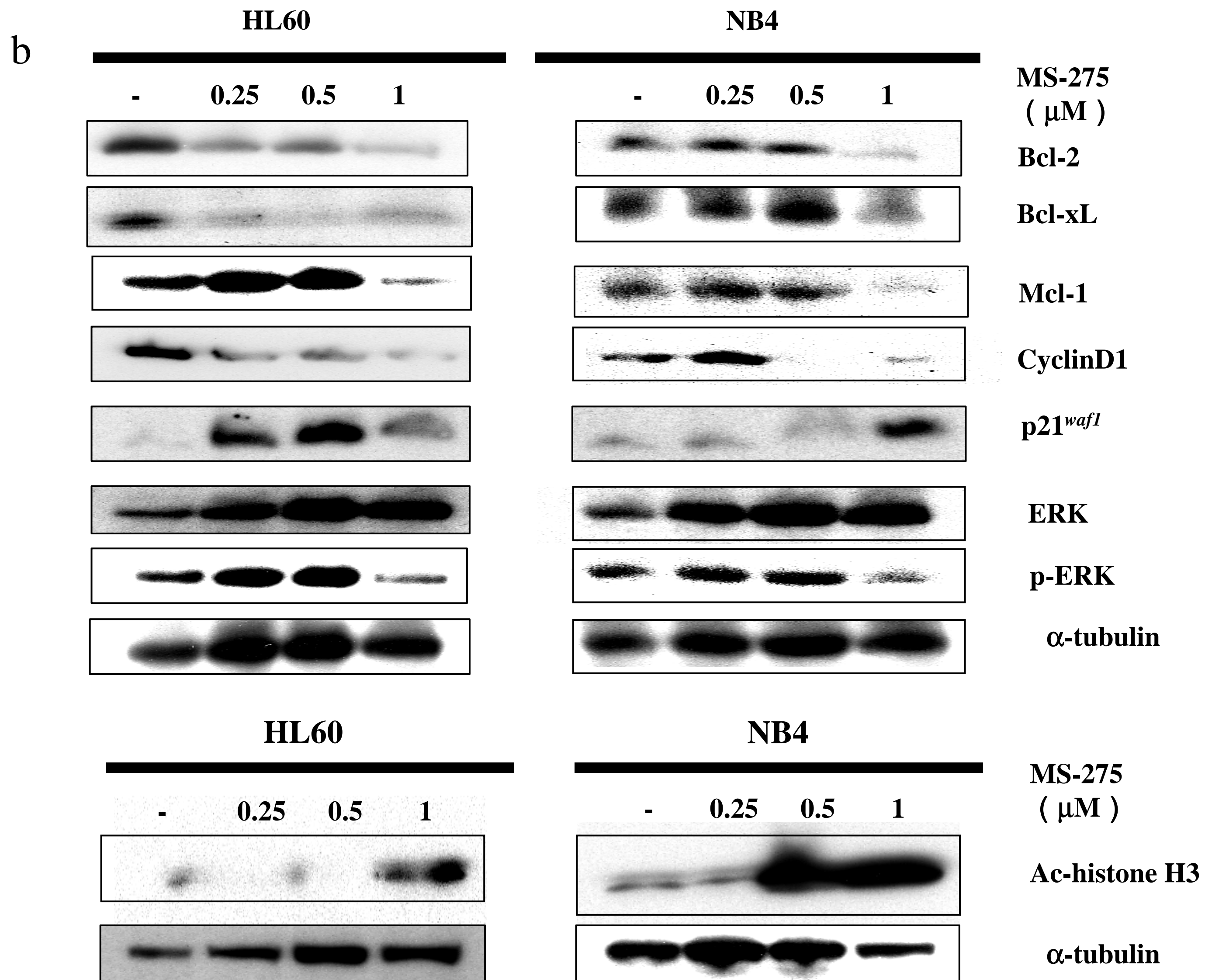

Figure 2 a

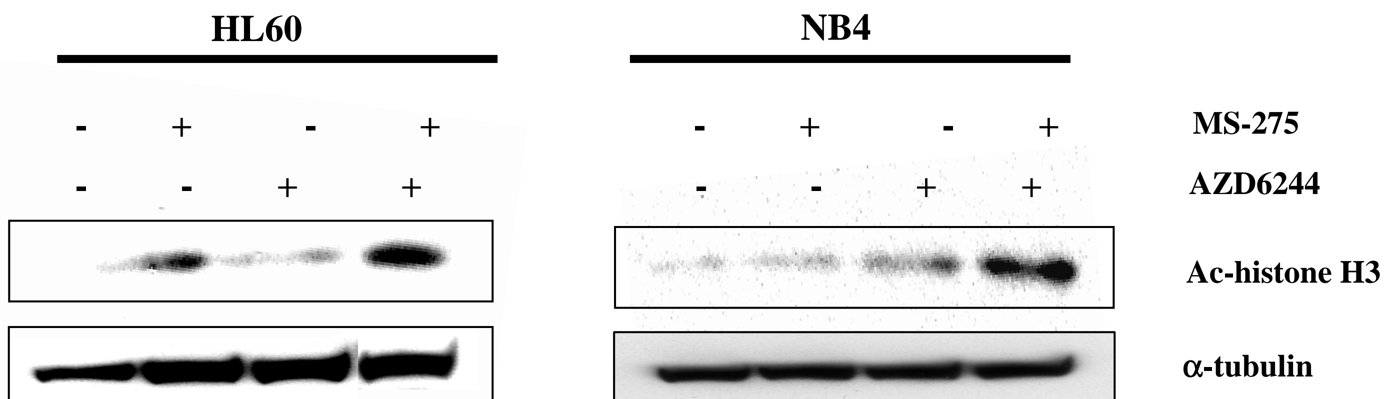

Figure 2 b

HL60

NB4

- + - +

- - + +

- + - +

- - + +

MS-275

AZD6244

ChIP

p21^{waf1} promoter

Input

p21^{waf1} promoter

Without ChIP

p21^{waf1} promoter

Table 1. Effect of MS-275 on freshly isolated acute leukemia cells

Pt. #	Age/Sex	FAB	WBC x10^9	% Blast	Genetic abnormalities	Source	IC_{50}	Previous Treatment
1	67/M	M2	38,700	54%	t(8;21)(q22;q22)	PB	0.05	No
2	57/M	M3	1,100	40%	t(15;17)(q22;q12)	BM	0.3	No
3	63/M	M4	79,400	69%	normal	PB	0.1	No
4	79/F	M4	34,200	50%	normal	PB	0.06	No
5	77/F	M7	2,000	38%	complex	PB	1	Yes
6	50/F	M3	6,300	89%	t(15;17)(q22;q12)	BM	0.06	No

The freshly isolated leukemia cells were cultured in the presence of various concentrations of MS-275 (0.03–1 μM).
After 2 days, cell proliferation was measured by tritiated thymidine uptake. The results were graphed and the concentration of MS-275 that induced 50% inhibition (IC_{50}) of thymidine-uptake was calculated from the dose-response curves.
BM, bone marrow; F, female; FAB, French–American-British (leukemia classification); M, male; PB, peripheral blood; Pt, patient; WBC, white blood cell.