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Effects of downregulated HDAC6 expression on the proliferation of lung cancer cells

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ABSTRACT

Histone deacetylase 6 (HDAC6) is a multifunctional, cytosolic protein deacetylase that primarily acts on α-tubulin. Here we report that stable knockdown of HDAC6 expression causes a decrease in the steady-state level of receptor tyrosine kinases, such as epidermal growth factor receptor (EGFR) and platelet-derived growth factor receptor α, in A549 lung cancer cells. The decreased levels of in EGFR in HDAC6-knockdown cells, which correlated with increased acetylation of microtubules, were due to increased turnover of EGFR protein. Despite the decrease in EGFR levels, A549 cells lacking functional HDAC6 appeared to grow normally, probably due to increased expression of extracellular signal-regulated kinases 1 and 2. Indeed, HDAC6-knockdown cells were more sensitive than control cells to the MEK inhibitor U0126. These results suggest that HDAC6 inhibitors combined with inhibitors of growth factor signaling may be useful as cancer therapy.

Histone deacetylase 6 (HDAC6) has been identified as a cytoplasmic deacetylase that associates with microtubules (MTs) and deacetylates α-tubulin [1–3]. Most mammalian cells possess two subsets of MTs: dynamic MTs with a half-life of minutes, and stable MTs with a half-life of several hours [4]. A subset of the stable MTs is highly acetylated at the Lys40 ε-amino group of α-tubulin [5,6]. Moreover, HDAC6-mediated α-tubulin deacetylation has been shown to destabilize dynamic MTs [2] and promote cell motility [1,7], a process that has been linked to the prognosis of breast cancer [7]. Additionally, the activity of such molecular motors as dynein and kinesin-1 on MTs has recently been shown to be mediated by MT acetylation [8,9]; MT acetylation promotes anterograde transport of the kinesin-1 cargo c-Jun N-terminal kinase-interacting protein 1 [8], whereas both anterograde and retrograde transport of brain-derived neurotrophic factor-containing vesicles has been shown to increase in response to HDAC6 inhibition [9].

HSP90 is another known substrate of HDAC6 [10,11]. The chaperone activity of HSP90 is partially activated by HDAC6-mediated deacetylation and inactivation of HDAC6 leads to hyperacetylation of HSP90 and instability of some of its client proteins, such as steroid receptors and signaling protein kinases [10,11]. HSP90 also regulates the maturation and stability of some receptor tyrosine kinases (RTKs), including platelet-derived growth factor receptor α (PDGFRα) [12], and it has recently been demonstrated that inhibition of HDAC6 promotes dissociation of HSP90 from vascular endothelial growth factor receptor 1 or 2 and a reduction in the steady-state levels of these receptors [13]. These lines of evidence demonstrate that HDAC6-mediated HSP90 deacetylation modulates the steady-state levels or maturation of the client RTKs.

Despite the extensive cellular roles of HDAC6, a recent study of HDAC6 knockout mice demonstrated that HDAC6 is dispensable for many major biological processes such as the cell cycle and growth signal transduction [14]. In this paper, we analyzed the effects of HDAC6 knockdown on cell growth using A549 lung carcinoma

Abbreviations: HDAC6, histone deacetylase 6 MT, microtubule RTK, receptor tyrosine kinase EGFR, epidermal growth factor receptor PDGFRα, platelet-derived growth factor receptor α ERK, extracellular signal-regulated kinase

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cells. Similar to the embryonic fibroblasts of HDAC6-null mice, HDAC6-deficient A549 cells showed normal cell growth. The levels of both epidermal growth factor receptor (EGFR) and PDGFRα, however, were reduced in response to the knockdown of HDAC6 expression. Interestingly, upregulation of the levels of extracellular signal-regulated kinases (ERKs) 1 and 2, which are downstream effectors of EGF signaling, was observed in HDAC6-silenced cells; this may provide a compensatory mechanism for survival in cancer cells lacking HDAC6.

Materials and methods

Reagents. Mouse anti-α-tubulin (B-5-1-2) and anti-acetylated (Ac-) α-tubulin (6-11B-1) antibodies were purchased from Sigma–Aldrich. Rabbit anti-detyrosinated (Glu-) α-tubulin antibodies were a generous gift from Dr. T.H. MacRae (Department of Biology, Dalhousie University, Canada). Mouse anti-ubiquitin (P4D1), anti-EGFR (528, for immunoprecipitation), rabbit anti-EGFR (1005, for Western blotting), anti-PDGFRα, (951), anti-HDAC6 (H-300), anti-cyclin A (H-432), anti-CDK2 (M2), and anti-CDK4 (H-22) antibodies were obtained from Santa Cruz Biotechnology. Mouse anti-phospho-Tyr (PY20) and anti-$p27^{Kip1}$ (57) antibodies were purchased from BD Biosciences. Mouse anti-phospho-p44/42 MAPK (phospho-ERK1/2, 9106) and rabbit anti-p44/42 MAPK (ERK1/2, 9102) antibodies were obtained from Cell Signaling Technology. Mouse anti-transferrin receptor antibodies (H68.4) were purchased from Zymed Laboratories. Cycloheximide and AG1478 were obtained from Sigma-Aldrich and Calbiochem, respectively.

Cell culture and transfection. A549 human lung carcinoma cells were cultured in DMEM supplemented with 10% (v/v) heat-inactivated fetal calf serum (FCS). An established A549 cell line expressing small-interfering RNA specific for HDAC6 was described previously [15].

Immunoprecipitation and Western blotting. Cells were lysed in buffer containing 10 mM Tris–HCl (pH 7.4), 0.5% (w/v) Triton X-100, 154 mM NaCl, and a protease inhibitor cocktail (Complete, Roche). The extracts were centrifuged for 20 min at 12,000*g* and the supernatants were used as cell lysates. The cell lysates were incubated with the indicated antibodies for 2 h at 4 °C. The immune complexes were precipitated with protein A/G-agarose (Santa Cruz Biotechnology) and washed thoroughly. To detect the proteins on the Western blots, the samples were subjected to immunodetection using the appropriate primary antibodies. Proteins were visualized using horseradish peroxidase-linked secondary antibodies and an enhanced chemiluminescence kit (GE Healthcare).

For EGF stimulation, cells were placed in 1% bovine serum albumin/DMEM for 12 h, incubated with 200 ng/ml recombinant human EGF (Sigma) for various lengths of time, washed with PBS, and then lysed as described above.

Quantitative RT-PCR. RNA was isolated using TRIzol reagent (Invitrogen) and reverse transcription was performed using a TaKaRa RNA PCR kit (AMV). Real-time PCRs were performed using qPCR MasterMix Plus for SYBR Green I Low ROX (Nippon Gene) and real-time detection of PCR products was carried out using an Applied Biosystems 7500 Real-time PCR system. GAPDH was used as the internal control to normalize the data. Primers specific for ERK1, ERK2, and GAPDH were as follows: ERK1 sense, 5′-CTCGCGTGGCCATCAAG-3′; ERK1 antisense, 5′-GCGTGCGCTGGC AGTAG-3′; ERK2 sense, 5′-CGTGACCTCAAGCCTTCCA-3′; ERK2 antisense, 5′-GGCCAAAGTCACAGATCTTGAGA-3′; GAPDH sense, 5′-GCC AAGGTCATCCATGACAACT-3′; and GAPDH antisense, 5′-GAGGGG CCATCCACAGTCTT-3′.

Cell cycle analysis and proliferation assay. For the cell cycle analysis, cultured cells were fixed in 70% ethanol/PBS, pelleted and resuspended in buffer containing 100 μg/ml RNase A and 0.01 mg/ml propidium iodide. The distribution of the cells throughout the cell cycle was analyzed using an EPICS XL flow cytometer (Beckman Coulter).

To monitor proliferation, cells were seeded in triplicate into 96-well plates at a density of 5000 cells/well. After 24 h in DMEM with 0.2% FCS, the cells were exposed to 10% FCS, and the viable cells were counted at the indicated time points using a XTT Cell Proliferation Kit II (Roche). Plates were analyzed in a microtiter plate reader at 492 nm with a reference wavelength of 620 nm. The sensitivity of cells to U0126 (Wako Pure Chemicals) was estimated using the same kit. Cells were incubated with U0126 at various concentrations for 4 days, and cell viability was measured. The IC_{50} value was calculated using the 50% cell survival rate compared with a control sample that did not contain the drug. Experiments were performed in triplicate.

Results and discussion

HDAC6 regulates the polymerization of microtubules

Using a pharmacologic approach, we previously demonstrated that HDAC6 promotes instability of a dynamic pool of MTs [2]. To confirm this using an alternative approach, we first examined the polymerized/depolymerized ratio of MTs in cells in which HDAC6 expression was stably knocked down (HDAC6-KD cells). As shown in Fig. 1A, the level of Ac-α-tubulin was significantly increased in HDAC6-KD cells compared to control cells (pS cells), whereas cells stably expressing wild-type HDAC6 (WT OP cells) reduced the degree of acetylation to an almost undetectable level. The ratios of polymerized MT versus depolymerized MTs in pS cells, HDAC6-KD cells, WT OP cells, and cells stably expressing a catalytically inactive mutant of HDAC6 (DC OP cells) were determined using a cell fractionation method, as reported previously [2]. Interestingly, HDAC6-KD cells showed an approximately three-fold higher level of the polymer than that in pS cells, whereas the level in WT OP cells was approximately half of the pS level (Fig. 1B). DC OP cells showed a similar level of the polymer as that observed in pS cells. These results indicate that the enzymatically active HDAC6 reduced the bulk level of polymerized MTs. Furthermore, the amount of Glu-α-tubulin, a marker for stable MTs [6], was markedly increased in HDAC6-KD cells, which supports the idea that HDAC6 regulates MT stability (Fig. 1C).

HDAC6 deficiency promotes RTK instability in A549 cells

Because MT dynamics are closely related to cell proliferation, HDAC6-mediated deacetylation of MTs may affect cell growth. To gain insights into the relevance of HDAC6 for cancer cell proliferation, we analyzed the role of HDAC6 in growth control using HDAC6-KD cells. Flow cytometric analysis showed that knockdown of HDAC6 expression did not affect the distribution of A549 cells among the various phases of the cell cycle (Fig. 2A). Indeed, the overall growth rate was similar in the pS and HDAC6-KD cells (Fig. 2B). The expression levels of such cell-cycle regulators as CDK2, CDK4, cyclin A, and $p27^{Kip1}$ were almost unchanged in the HDAC6-KD cells (Fig. 2C). The EGFR level, however, was markedly reduced in the HDAC6-KD cells (Fig. 2C). To test whether the decreased EGFR level correlated with the reduced level of HDAC6, we compared the amounts of HDAC6 and EGFR in the early passages of the HDAC6-KD cells. As the passage number increased, the HDAC6 level in HDAC6-KD cells gradually decreased, whereas the Ac-α-tubulin level increased (Fig. 2D). Importantly, the EGFR level also decreased in a passage number-dependent manner. On the other hand, the level of transferrin receptor, which is constitutively recycled [16], was constant throughout the passages. Moreover, the level of PDGFRα, which is known to be expressed in A549 cells [17], was also decreased

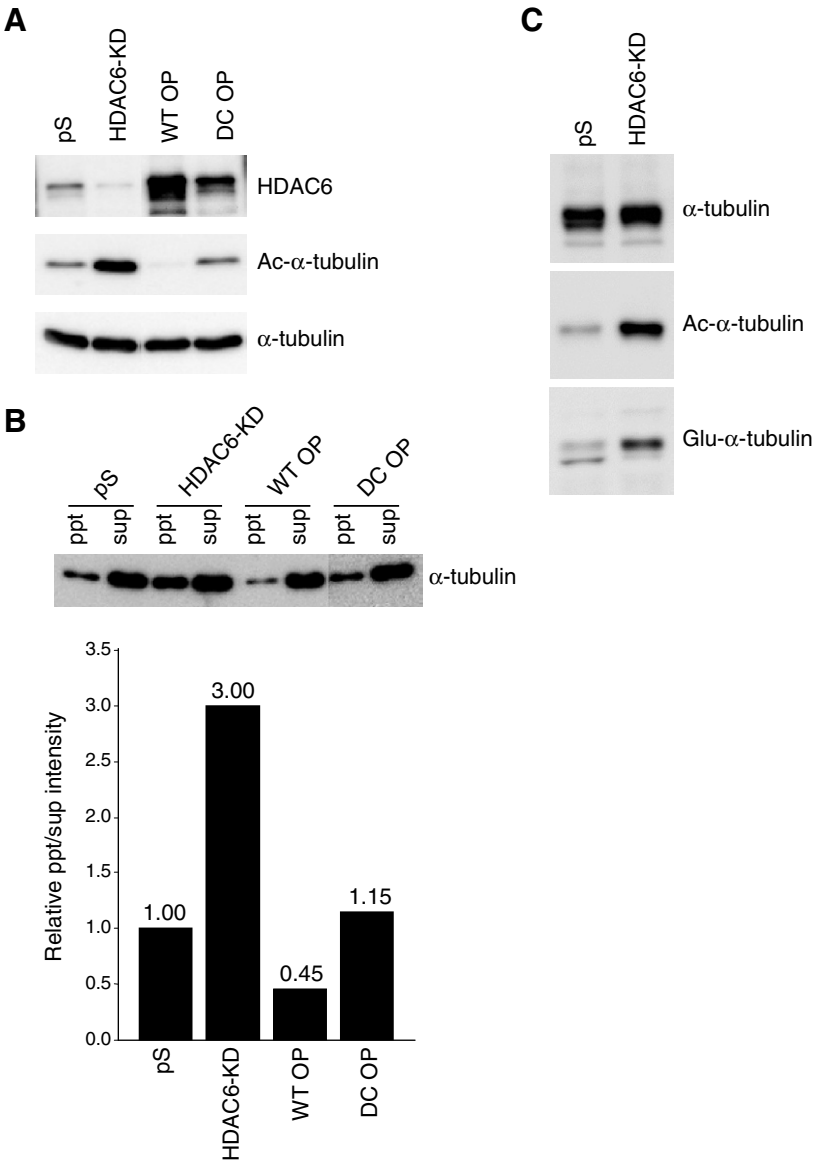

Fig. 1. Increase in polymerized microtubules in HDAC6-depleted cells. (A) Increase in acetylated α-tubulin (Ac-α-tubulin) in HDAC6-depleted cells. Whole cell lysates from control (pS) cells, stable HDAC6-knockdown (HDAC6-KD) cells, wild-type cells overexpressing HDAC6 (WT OP), or cells overexpressing catalytically inactive HDAC6 (DC OP) were immunoblotted for the indicated proteins. (B) Increase in polymerized microtubules due to the loss of HDAC6. Cells were treated with 5 μM paclitaxel and lysed. Total cell lysates were separated into the precipitates and supernatants by centrifugation at 16,000*g* and the fractions were immunoblotted for α-tubulin. The intensity of each band was quantified using densitometry (experiments were performed in triplicate) and the precipitate/supernatant ratios were determined. (C) Increase in detyrosinated (Glu-) α-tubulin in HDAC6-KD cells.

in the HDAC6-KD cells. To test whether the decreased level of EGFR in HDAC6-KD cells was due to protein degradation, we measured the half-lives of the EGFR protein in the pS and HDAC6-KD cells. As shown in Fig. 2E, the half-life of the EGFR protein in the HDAC6-KD cells was shortened to approximately half of that in the pS cells (pS, $t_{1/2}$ = 16.2 h; KD, $t_{1/2}$ = 8.6 h), indicating that the decreased EGFR levels in HDAC6-KD cells are, at least in part, due to the instability of the EGFR protein. AG1478, a potent and selective EGFR kinase inhibitor, rescued EGFR from the degradation induced by EGF stimulation in HDAC6-KD cells (Fig. 2F). This result suggested that the downregulation of EGFR levels in HDAC6-KD cells is ligand-dependent.

There are two possible mechanisms by which RTK levels may have been downregulated in HDAC6-KD cells. First, because the stability of such RTKs as PDGFRα and EGFR has been shown to be reduced by HSP90 inhibition [12,18–20], the decrease in RTK levels in HDAC6-KD cells might be induced by increased acetylation of HSP90, which reduces the chaperon activity. Second, increased stability of the MTs may contribute to the decrease in RTK levels in HDAC6-KD cells; increased MT stability may accelerate the lysosomal degradation of the endocytosed RTKs through a facilitation of MT-based endosomal traffic. This idea is also supported by recent observations that acetylated MTs function as an effective track for vesicle transport in the endocytic pathway [8,9]. Although the mechanism underlying the reduced EGFR levels is still unclear, we wanted to address how the A549 cells can grow normally in the absence of wild-type levels of EGFR.

Compensation for the decreased RTK levels by ERK1/2

Most lung cancer cells require EGF for their growth, which is why EGF receptor kinase inhibitors such as ZD1839 (Iressa) show clinical efficacy [21,22]. Despite decreased EGFR expression, however, cell-cycle progression and the proliferation rate

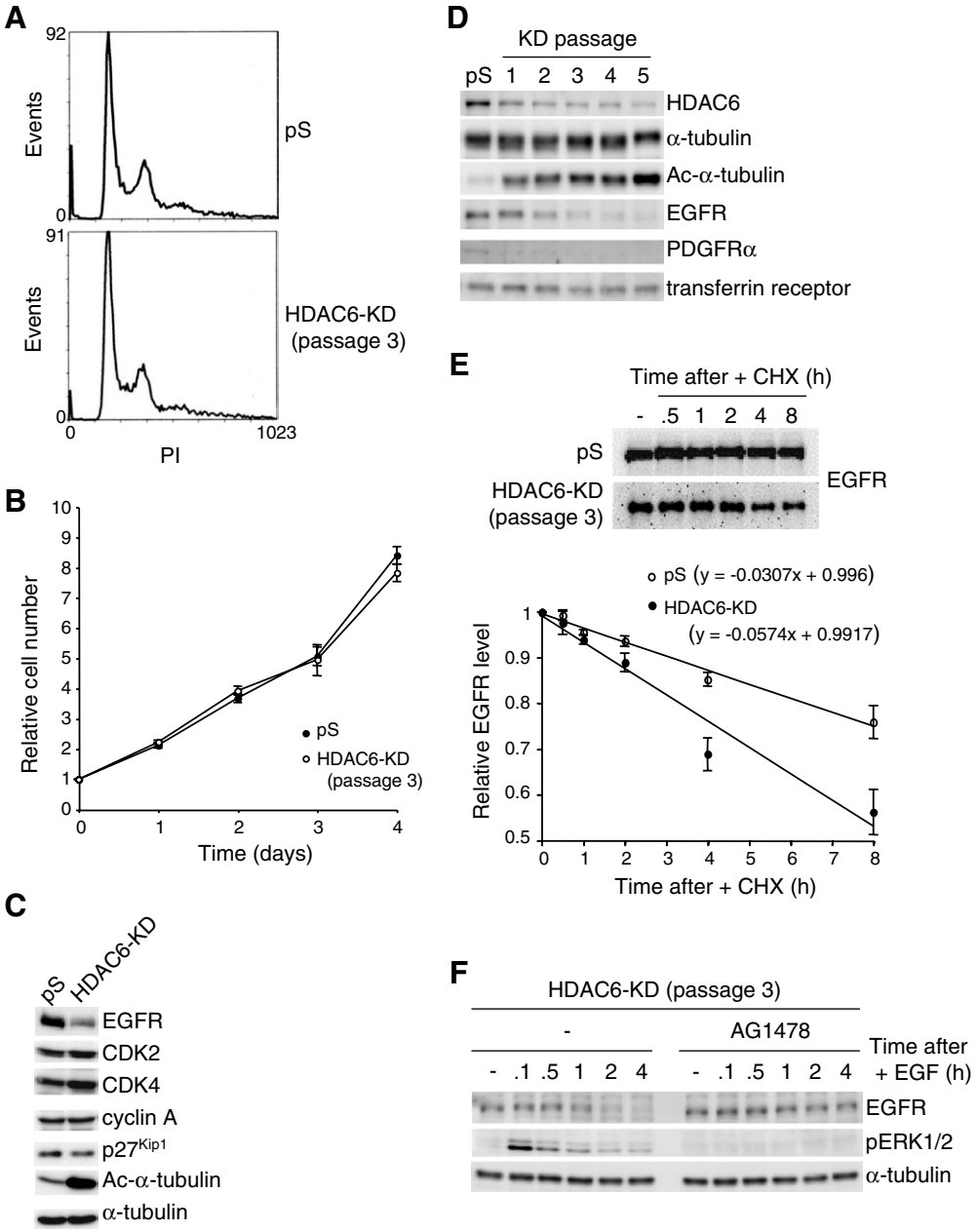

Fig. 2. Normal cell proliferation in HDAC6-depleted cells despite decreased RTK levels. (A) Cell cycle distribution of HDAC6-KD cells. A total of 10,000 cells were analyzed from each sample. (B) Proliferation profiles of HDAC6-KD cells. Data are means ± SD from three independent assays. (C) Levels of cell cycle-related proteins in HDAC6-KD cells. Whole cell lysates from pS or HDAC6-KD (passage 3) cells were immunoblotted for the indicated proteins. (D) Decrease in EGFR and PDGFRα levels in HDAC6-KD cells. Cells were harvested at the indicated serial passage and whole cell lysates were immunoblotted for the indicated proteins. (E) Rapid turnover of EGFR in HDAC6-KD cells. Cells were treated with cycloheximide (CHX, 1 μg/ml) for the indicated time. Whole cell lysates were immunoblotted for EGFR (upper panel, a representative image is shown) and the intensity of the EGFR band at each time point was normalized to that of the α-tubulin band (lower panel, means ± SD from three independent experiments are shown). (F) Ligand-dependent degradation of EGFR in HDAC6-KD cells. Cells were subjected to mock treatment (DMSO) or 0.5 μM AG1478 for 2 h and then stimulated with EGF (200 ng/ml) for the indicated time. Western blotting of whole cell lysates was performed for the indicated proteins.

were unaffected by knockdown of HDAC6 expression (Fig. 2A and B). To examine the underlying mechanism, we examined the effects of HDAC6 knockdown on the autophosphorylation and ubiquitination of EGFR to monitor EGFR activation, and on the expression and phosphorylation of ERK1/2, downstream effectors in the EGF signaling pathway [23]. Immunoprecipitation experiments revealed that EGF-dependent tyrosine phosphorylation, following multiple monoubiquitination signals, and downregulation of EGFR levels were induced by EGF stimulation in both control and knockdown cells, although the resulting EGFR level in HDAC6-KD cells was much lower (Fig. 3A). Surprisingly, the ERK1/2 levels were much higher in HDAC6-KD cells than in pS cells. Moreover, more phosphorylation of ERK1/2 was observed in HDAC6-KD cells (Fig. 3A). The increase in ERK1/2 levels may have been due to increased expression rather than decreased degradation, because the half-lives of ERK1/2 proteins in HDAC6-KD cells was almost the same as that observed in pS cells (Fig. 3B) and the mRNA levels of ERK1/2 in the HDAC6-KD cells were approximately 1.5-fold higher than those detected in pS cells (Fig. 3C). These results strongly suggest that increased expression of ERK1/2 compensates for the decreased EGFR signaling in the HDAC6-KD cells. It is likely that the weak upstream signaling activity required to activate ERKs would be readily reduced below a threshold level by a signaling inhibitor. Indeed,

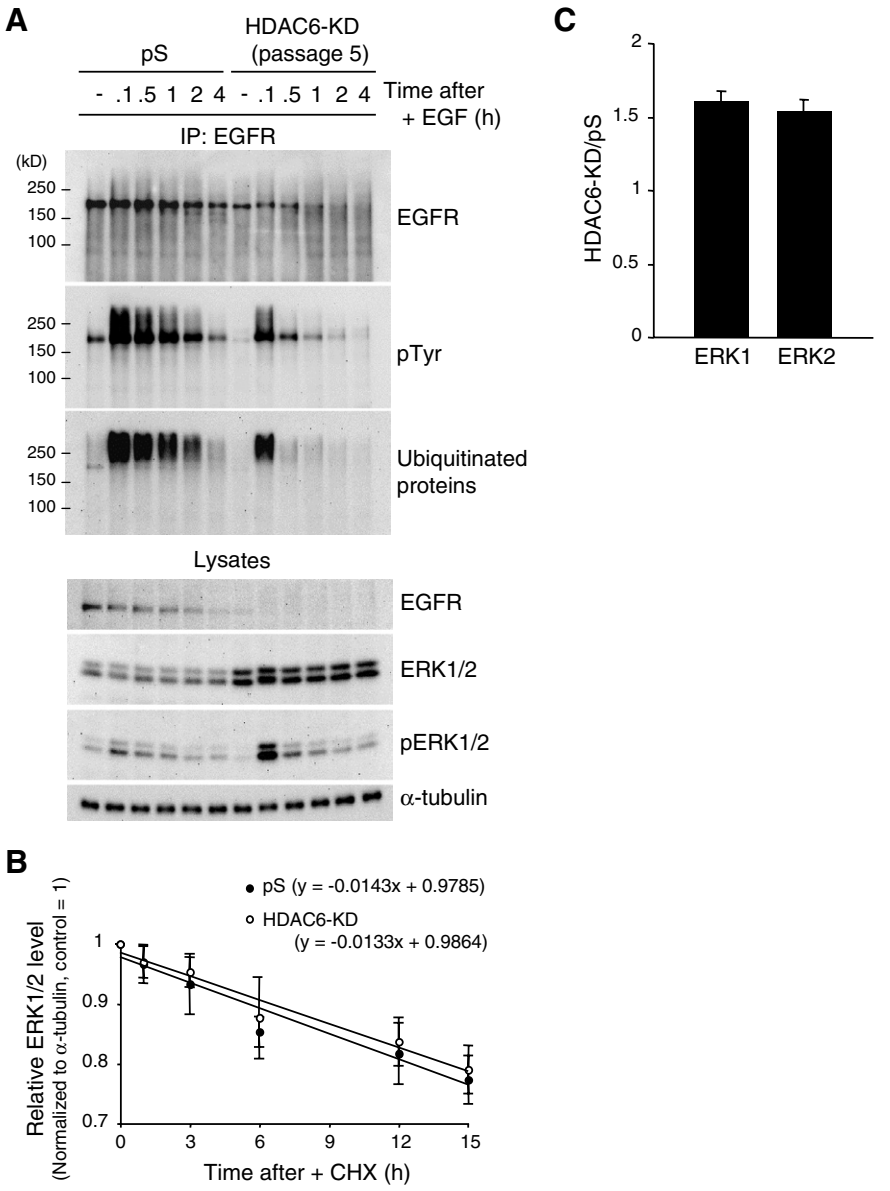

Fig. 3. Compensation for decreased EGF signaling by ERK1/2 in HDAC6-depleted cells. (A) Cells were stimulated with EGF (200 ng/ml) for the indicated time. Western blotting for the indicated proteins in both whole cell lysates and the immunoprecipitates from the lysates obtained with anti-EGFR antibodies are shown. (B) A comparison of the turnover of ERK1/2 between pS and HDAC6-KD (passage 5) cells. Cells were treated with CHX (1 μg/ml) for the indicated time. Whole cell lysates were immunoblotted for ERK1/2 and the intensity of the ERK1/2 band at each time point was normalized to that of the α-tubulin band. Data are means ± SD from three independent experiments. (C) A comparison of ERK1/2 mRNA levels in pS and HDAC6-KD (passage 5) cells using quantitative RT-PCRs.

compared with the pS cells, HDAC6-KD cells were approximately twice as sensitive to the MEK inhibitor U0126 (Table 1). A similar result was observed with PD98059 (data not shown), which supports the idea that proliferation of the knockdown cells was more ERK-signaling-dependent than that of the control cells.

Dysregulation of RTKs, including EGFR, plays an important and causative role in tumorigenesis in a wide variety of cancers [24]. The novel function of HDAC6 uncovered in this study may contribute to the clinical benefits of HDAC6 inhibitors for the treatment of many cancers involving activated RTKs. Depletion of HDAC6 alone, however, did not affect cell-cycle progression in A549 cells. Their apparently normal growth may be due to compensation for the decreased RTK signaling by increased expression and activity of ERK1/2. Indeed, A549 cells in which HDAC6 expression was stably knocked down were more sensitive to a MEK inhibitor. Thus, using inhibitors of downstream signaling molecules such as MEK together with HDAC6 inhibitors may be a promising therapeutic strategy. Indeed, coadministration of HDAC inhibitors and MEK inhibitors has been reported to cause a synergistic induction of apoptosis in Bcr/Abl$^+$ human leukemia cells [25,26]. Further studies are needed to confirm the efficacy of combination therapy consisting of HDAC6 inhibitors with RTK signaling inhibitors.

Table 1
A comparison of the sensitivities of HDAC6-depleted cells and control cells to U0126

A549 cell line	pS	HDAC6-KD
IC_{50} (μM)	19.2	9.7

The IC_{50} value was calculated using the 50% cell survival rate compared with a control sample that was not treated with the drug.

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References

[1] C. Hubbert, A. Guardiola, R. Shao, Y. Kawaguchi, A. Ito, A. Nixon, M. Yoshida, X-F. Wang, T-P. Yao, HDAC6 is a microtubule-associated deacetylase, Nature 417 (2002) 455–458.
[2] A. Matsuyama, T. Shimazu, Y. Sumida, A. Saito, Y. Yoshimatsu, D. Seigneurin-Berny, H. Osada, Y. Komatsu, N. Nishino, S. Khochbin, S. Horinouchi, M. Yoshida, In vivo destabilization of dynamic microtubules by HDAC6-mediated deacetylation, EMBO J. 21 (2002) 6820–6831.
[3] Y. Zhang, N. Li, C. Caron, G. Matthias, D. Hess, S. Khochbin, P. Matthias, HDAC-6 interacts with and deacetylates tubulin and microtubules in vivo, EMBO J. 22 (2003) 1168–1179.
[4] E. Schulze, D.J. Asai, J.C. Bulinski, M. Kirschner, Posttranslational modification and microtubule stability, J. Cell Biol. 105 (1987) 2167–2177.
[5] G. Piperno, M. LeDizet, X-J. Chang, Microtubules containing acetylated α-tubulin in mammalian cells in culture, J. Cell Biol. 104 (1987) 289–302.
[6] S. Westermann, K. Weber, Post-translational modifications regulate microtubule function, Nat. Rev. Mol. Cell Biol. 4 (2003) 938–947.
[7] S. Saji, M. Kawakami, S-I. Hayashi, N. Yoshida, M. Hirose, S-I. Horiguchi, A. Itoh, N. Funata, S.L. Schreiber, M. Yoshida, M. Toi, Significance of HDAC6 regulation via estrogen signaling for cell motility and prognosis in estrogen receptor-positive breast cancer, Oncogene 24 (2005) 4531–4539.
[8] N.A. Reed, D. Cai, T.L. Blasius, G.T. Jih, E. Meyhofer, J. Gaertig, K.J. Verhey, Microtubule acetylation promotes kinesin-1 binding and transport, Curr. Biol. 16 (2006) 2166–2172.
[9] J.P. Dompierre, J.D. Godin, B.C. Charrin, F.P. Cordelières, S.J. King, S. Humbert, F. Saudou, Histone deacetylase 6 inhibition compensates for the transport deficit in Huntington's disease by increasing tubulin acetylation, J. Neurosci. 27 (2007) 3571–3583.
[10] J.J. Kovacs, P.J.M. Murphy, S. Gaillard, X. Zhao, J-T. Wu, C.V. Nicchitta, M. Yoshida, D.O. Toft, W.B. Pratt, T-P. Yao, HDAC6 regulates Hsp90 acetylation and chaperone-dependent activation of glucocorticoid receptor, Mol. Cell 18 (2005) 601–607.
[11] P. Bali, M. Pranpat, J. Bradner, M. Balasis, W. Fiskus, F. Guo, K. Rocha, S. Kumaraswamy, S. Boyapalle, P. Atadja, E. Seto, K. Bhalla, Inhibition of histone deacetylase 6 acetylates and disrupts the chaperone function of heat shock protein 90: a novel basis of antileukemia activity of histone deacetylase inhibitors, J. Biol. Chem. 280 (2005) 26729–26734.
[12] D. Matei, M. Satpathy, L. Cao, Y-C. Lai, H. Nakshatri, D.B. Donner, The platelet-derived growth factor receptor α is destabilized by geldanamycins in cancer cells, J. Biol. Chem. 282 (2007) 445–453.
[13] J.-H. Park, S.-H. Kim, M.-C. Choi, J. Lee, D.-Y. Oh, S.-A. Im, Y.-J. Bang, T.-Y. Kim, Class II histone deacetylases play pivotal roles in heat shock protein 90-mediated proteasomal degradation of vascular endothelial growth factor receptors, Biochem. Biophys. Res. Commun. 368 (2008) 318–322.
[14] Y. Zhang, S.-H. Kwon, T. Yamaguchi, F. Cubizolles, S. Rousseaux, M. Kneissel, C. Cao, N. Li, H.-L. Cheng, K. Chua, D. Lombard, A. Mizeracki, G. Matthias, F.W. Alt, S. Khochbin, P. Matthias, Mice lacking histone deacetylase 6 have hyperacetylated tubulin but are viable and develop normally, Mol. Cell. Biol. 28 (2008) 1688–1701.
[15] Y. Kawaguchi, J.J. Kovacs, A. McLaurin, J.M. Vance, A. Ito, T.-P. Yao, The deacetylase HDAC6 regulates aggresome formation and cell viability in response to misfolded protein stress, Cell 115 (2003) 727–738.
[16] F.R. Maxfield, T.E. McGraw, Endocytic recycling, Nat. Rev. Mol. Cell Biol. 5 (2004) 121–132.
[17] P. Zhang, W.Y. Gao, S. Turner, B.S. Ducatman, Gleevec (STI-571) inhibits lung cancer cell growth (A549) and potentiates the cisplatin effect in vitro, Mol. Cancer 2 (2003) 1.
[18] W. Xu, E. Mimnaugh, M.F.N. Rosser, C. Nicchitta, M. Marcu, Y. Yarden, L. Neckers, Sensitivity of mature ErbB2 to geldanamycin is conferred by its kinase domain and is mediated by the chaperone protein Hsp90, J. Biol. Chem. 276 (2001) 3702–3708.
[19] T. Shimamura, A.M. Lowell, J.A. Engelman, G.I. Shapiro, Epidermal growth factor receptors harboring kinase domain mutations associate with the heat shock protein 90 chaperone and are destabilized following exposure to geldanamycins, Cancer Res. 65 (2005) 6401–6408.
[20] S. Yang, S. Qu, M. Perez-Tores, A. Sawai, N. Rosen, D.B. Solit, C.L. Arteaga, Association with HSP90 inhibits Cbl-mediated down-regulation of mutant epidermal growth factor receptors, Cancer Res. 66 (2006) 6990–6997.
[21] M. Tiseo, M. Loprevite, A. Ardizzoni, Epidermal growth factor receptor inhibitors: a new prospective in the treatment of lung cancer, Curr. Med. Chem. Anticancer Agents 4 (2004) 139–148.
[22] P.M. Harari, Epidermal growth factor receptor inhibition strategies in oncology, Endocr. Relat. Cancer 11 (2004) 689–708.
[23] G.L. Johnson, R. Lapadat, Mitogen-activated protein kinase pathways mediated by ERK, JNK, and p38 protein kinases, Science 298 (2002) 1911–1912.
[24] P. Blume-Jensen, T. Hunter, Oncogenic kinase signalling, Nature 411 (2001) 355–365.
[25] C. Yu, G. Dasmahapatra, P. Dent, S. Grant, Synergistic interactions between MEK1/2 and histone deacetylase inhibitors in BCR/ABL+ human leukemia cells, Leukemia 19 (2005) 1579–1589.
[26] C. Yu, M. Subler, M. Rahmani, E. Reese, G. Krystal, D. Conrad, P. Dent, S. Grant, Induction of apoptosis in BCR/ABL+ cells by histone deacetylase inhibitors involves reciprocal effects on the RAF/MEK/ERK and JNK pathways, Cancer Biol. Ther. 2 (2003) 544–551.