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The Anticancer Effect of Histone Deacetylase Inhibitors and Combination with the Cytotoxic Agents in Lung Cancer Cells: Biological Analyses for Future Clinical Application

Rintaro Noro, Akihiko Miyanaga, Tsuneo Shimokawa, Hidehiko Kuribayashi, Hideaki Mizutani, Yuji Minegishi, Tetsuya Okano, Masahiro Seike, Chie Soeno, Kiyoko Kataoka, Kuniko Matsuda, Akinobu Yoshimura and Akihiko Gemma

Department of Pulmonary Medicine/Infection and Oncology, Nippon Medical School

Introduction

Even though a wide range of anticancer agents have been developed, many patients with advanced solid tumors still have a poor prognosis. For the treatment of advanced lung cancer, there are many anticancer agents in clinical use, such as cisplatin, carboplatin, docetaxel, paclitaxel, vinorelbine, gemicitabine, fluorouracil (5-FU) derivatives, and irinotecan. A number of combination therapy regimens employing platinum compounds have proven to be effective and are widely applied for the initial treatment of inoperative non-small cell lung cancer (NSCLC). In addition, docetaxel, pemetrexed, and epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors have been reported to be effective in the context of second-line chemotherapy for NSCLC. However, the effect of these therapies on improving patient survival remains far from satisfactory. It is consequently desirable to find more appropriate therapeutic opportunities for NSCLC. Recently, molecularly targeted therapy for cancer has made substantial progress. Histone deacetylase (HDAC) inhibitors have been shown to acetylate the nucleosomal histones of condensed chromatin, and cause the reactivation of genes silenced by hyperacetylating histones. HDAC inhibitors, including suberoylanilide hydroxamic acid (SAHA), have demonstrated therapeutic benefits as monotherapy in hematologic, breast, and bladder malignancies, as well as mesothelioma and NSCLC, without evidence of severe adverse events. Recently, it has been demonstrated that combinations of HDAC inhibitors with well-established chemotherapeutic agents have synergistic antitumor effects via the modulation of biomarkers by HDAC inhibitors. We clarified the predictive markers to select patients receiving this treatment and found a new strategy against lung cancer by using the anticancer drug fluorouracil.

Method

To assess the potential for HDAC-inhibitor-based treatment in NSCLC, we analyzed the antitumor effects of trichostatin A (TSA) and SAHA (vorinostat) in a panel of 16 NSCLC cell lines via 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. To identify a molecular model of sensitivity to HDAC inhibitor treatment in NSCLC, we conducted a gene expression profiling study with cDNA arrays on the same set of cell lines and related the cytotoxic activity of TSA to corresponding gene expression patterns by means of a modified National Cancer Institute program. In addition, pathway analysis was done with Pathway Architect software

Table 1 Factors associated with TSA sensitivity based on expression profiles, sensitivity, and pathway analyses in the 16 NSCLC cell line panel and their functions

Probe Set ID Correlation	Gene Symbol	Genes incorporated by pathway analysis	coefficients
1. 201064_s_at	*PABPC4*		
2. 201737_s_at	*MARCH6*		
3. 209339_at	*SIAH2*		
4. 212887_at	*SEC23A*	+	−0.734
5. 214857_at	*C10orf95*		
6. 217100_s_at	*UBXD7*		
7. 201762_s_at	*PSME2*	+	−0.683
8. 201919_at	*SLC25A36*		
9. 201993_x_at	*HNRPDL*	+	0.678
10. 202731_at	*PDCD4*	+	0.724
11. 208799_at	*PSMB5*	+	−0.688
12. 208912_s_at	*CNP*		
13. 209149_s_at	*TM9SF1*	+	−0.672
14. 209150_s_at	*TM9SF1*	+	−0.672
15. 210519_s_at	*NQO1*	+	−0.690
16. 211730_s_at	*POLR2L*		
17. 212082_s_at	*MYL6*	+	−0.718
18. 219717_at	*FLJ20280*		
19. 220200_s_at	*SETD8*		

Miyanaga A *et al*. Mol Cancer Ther 2008; 7 (7). 1923-30

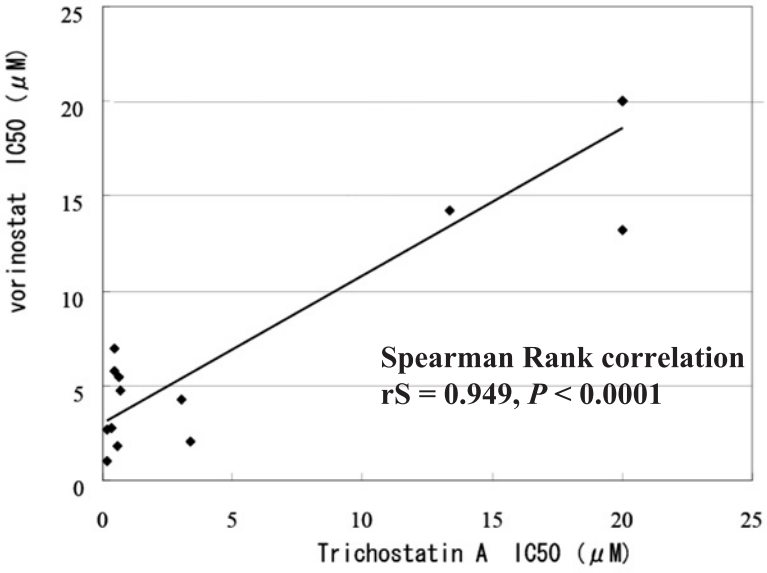

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Fig. 1 The correlation between the responsiveness to TSA and SAHA in a panel of 16 NSCLC cell lines

(Stratagene). The data from the listed genes were used to build a support vector machine model with ArrayAssist software (Stratagene) to predict the drug response (IC50).

Using the MTT assay, we analyzed the growth-inhibitory effect of 5-FU/S-1 and SAHA against lung cancer cells. The mRNA and protein expressions of Thymidylate synthase (TS), Dihydropyrimidine dehydrogenase (DPD) and Orotate phosphoribosyltransferase (OPRT), which are metabolites of 5-FU, were analyzed in these

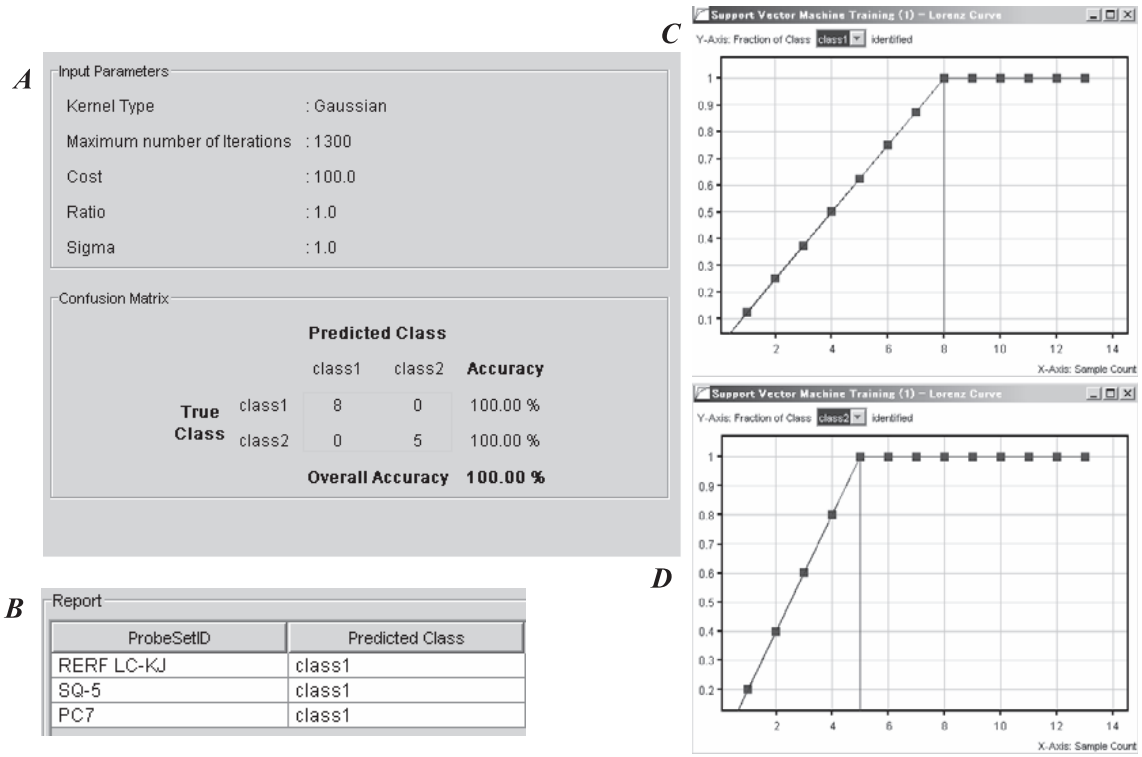

Fig. 2 Building a support vector machine algorithm model
Class 1: sensitive cell lines Class 2: resistant cell lines

cells. In order to clarify the mechanism of the synergistic effect of SAHA and 5-FU, we examined the change of the 5-FU metabolism expressions and their regulator including $p21^{waf1/cip1}$ and Rb-E2F1 pathway.

Results

Both TSA and SAHA showed strong antitumor activities in 50% of NSCLC cell lines, suggesting the need for the use of predictive markers to select patients receiving this treatment. There was a strong correlation between the responsiveness to TSA and SAHA ($P<0.0001$; **Fig. 1**). We used 9 genes, which were identified with gene-drug sensitivity correlation and pathway analysis (**Table 1**), to build a support vector machine algorithm model (**Fig. 2**), by which sensitive cell lines were distinguished from resistant cell lines. The prediction performance of the support vector machine model was validated with an additional 9 cell lines, resulting in a predictive value of 100% with respect to determining response to TSA and SAHA.

S-1, a novel oral fluorouracil anticancer drug, has been developed for clinical use against NSCLC in Japan. Combined treatment with low-dosage SAHA enhanced 5-FU/S-1 mediated cytoxity and the synegistic effect in 5-FU-resistant cells. The mRNA and protein expressions of TS, DPD and OPRT were analyzed in these cells. TS expression correlated with 5-FU sensitivity in these cells.

Conclusion

In conclusion, our results suggest that HDAC inhibitors may be promising anticancer drugs for NSCLC and that the 9-gene classifier is useful for predicting the sensitivity of NSCLCs to HDAC inhibitors and may contribute to achieving individualized therapy for patients with NSCLC. SAHA enhanced S-1 sensitivity in lung cancer cells, and this combination therapy may be effective against lung cancer.