



Guangdong Medical University

Molecular mechanism by which chidamide reverses osimertinib resistance in lung adenocarcinoma through downregulation of ARSD expression

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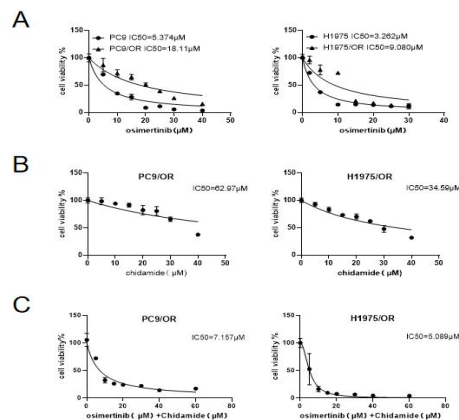
Introduction

ARSD has been implicated in the development of lung cancer and may function as a potential tumor suppressor or oncogenic factor. Altered expression of ARSD has been associated with cellular differentiation and various human diseases. However, the molecular mechanism underlying the role of ARSD in lung tumorigenesis remains unclear.

Methods

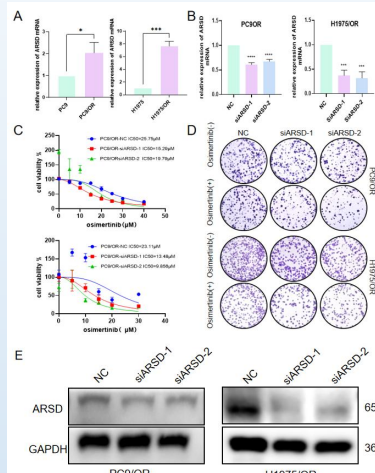
Here, we analyzed the function of ARSD in lung adenocarcinoma using xenografts, and non-small-cell lung cancer cell lines.

Results



Chidamide combined with osimertinib reduces the resistance index of resistant cells.

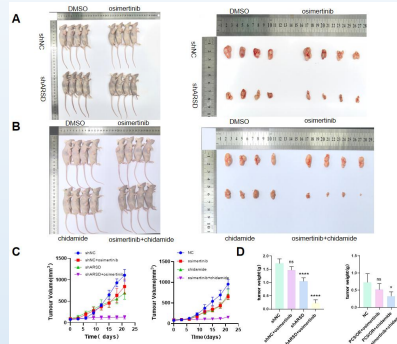
(A)PC9 and H1975 lung adenocarcinoma cells, representing different EGFR mutation backgrounds, were continuously exposed to increasing concentrations of osimertinib to establish resistant cell lines, PC9/OR and H1975/OR. (B)WST-1 assay showed that, compared with parental cells, both resistant cell lines exhibited significantly increased IC50 values and reduced sensitivity to osimertinib, indicating the successful establishment of stable osimertinib-resistant models.



Differential ARSD expression and reversal of osimertinib resistance in lung adenocarcinoma by ARSD knockdown.

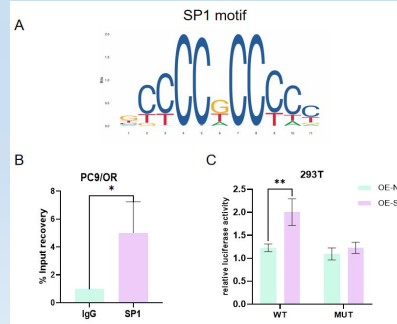
(A) Quantitative RT-PCR analysis of ARSD mRNA expression in parental lung adenocarcinoma cells and their osimertinib-resistant counterparts. ARSD expression was significantly increased in resistant cells compared with parental cells. (B) Knockdown efficiency of ARSD in PC9/OR and H1975/OR cells transfected with two independent siRNAs targeting ARSD, as determined by qRT-PCR. Negative control siRNA (NC) was used as the control. (C) WST assay of cell viability in PC9/OR and H1975/OR cells following ARSD knockdown and treatment with increasing concentrations of osimertinib for 72 h. ARSD silencing markedly enhanced the sensitivity of resistant cells to osimertinib, as reflected by reduced cell viability and decreased IC50 values. (D)ARSD knockdown significantly reduced clonogenic capacity and restored the inhibitory effect of osimertinib in resistant cells.

(E)Western blot analysis of ARSD protein expression in PC9/OR and H1975/OR cells after transfection with NC, siARSD-1, or siARSD-2. GAPDH was used as the loading control. The results confirmed that ARSD protein expression was effectively reduced after siRNA-mediated knockdown. (*, P < 0.05; **, P < 0.01; ****P < 0.001, ****P < 0.0001).



ARSD depletion or chidamide treatment enhances the antitumor effect of osimertinib in vivo.

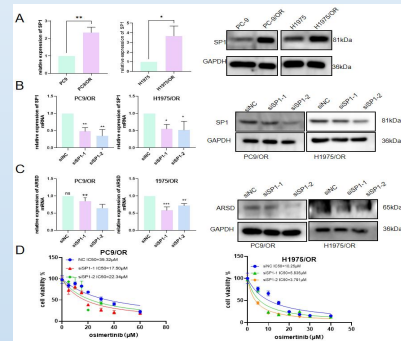
(A) Representative images of nude mice and excised tumors from xenografts formed by shNC or shARSD cells treated with DMSO or osimertinib. ARSD knockdown suppressed tumor growth and enhanced the antitumor effect of osimertinib. (B) Representative images of nude mice and excised tumors from xenografts treated with DMSO, osimertinib, chidamide, or osimertinib plus chidamide. The combination treatment showed the strongest tumor growth inhibition. (C) Tumor growth curves of the indicated xenograft groups. Both ARSD knockdown plus osimertinib and chidamide plus osimertinib markedly inhibited tumor growth compared with single treatment groups. (D) Final tumor weights of each group. ARSD knockdown or chidamide significantly enhanced the inhibitory effect of osimertinib on tumor growth. (* P < 0.05, ***P < 0.001, ****P < 0.0001; ns, not significant.)



Results

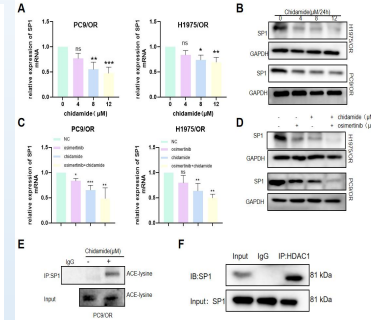
SP1 directly binds to the promoter region and transcriptionally activates ARSD.

(A) Sequence logo showing the predicted SP1-binding motif in the ARSD promoter region.(B) ChIP-qPCR analysis in PC9/OR cells showing the enrichment of SP1 at the ARSD promoter. Immunoprecipitation with an anti-SP1 antibody resulted in significantly higher promoter recovery than the IgG control, indicating direct binding of SP1 to the ARSD promoter. (C) Dual-luciferase reporter assay in 293T cells transfected with wild-type (WT) or mutant (MUT) ARSD promoter reporter constructs together with control vector (OE-NC) or SP1 overexpression plasmid (OE-SP1). SP1 overexpression significantly increased the luciferase activity of the WT promoter reporter, whereas mutation of the SP1-binding site abolished this effect, indicating that SP1 transcriptionally activates ARSD through this specific binding motif. (*, P < 0.05; **, P < 0.01)



Differential SP1 expression and reversal of osimertinib resistance in lung adenocarcinoma by SP1 knockdown.

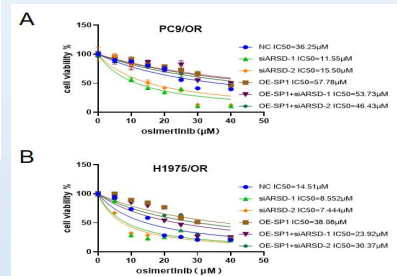
(A)Demonstrates that chidamide treatment dose-dependently reduces cell viability in PC9/OR cells, with significant inhibition observed at higher concentrations. (B) shows a similar dose-dependent reduction in cell viability in H1975/OR cells following chidamide treatment. (C)reveals that in PC9/OR cells, the combination of chidamide and osimertinib suppresses cell viability more effectively than either monotherapy, indicating a synergistic effect.(D) further confirms that in H1975/OR cells, the combined treatment also exhibits superior inhibition of cell viability compared to single agents alone. (*, P < 0.05; **, P < 0.01, ****P < 0.0001).



Chidamide affects SP1 expression through acetylation modification, thereby promoting drug resistance, and the deacetylase HDAC1 interacts with SP1.

(A) In PC9/OR cells, chidamide treatment dose-dependently reduces the relative expression of both SP1 mRNA and HSP70 mRNA.(B) Western blot analysis shows that chidamide treatment (increasing concentrations) decreases SP1 protein expression in both PC9/OR and H1975/OR cells, with GAPDH as a loading control.(C) In both PC9/OR and H1975/OR cells, the combination of chidamide and osimertinib suppresses SP1 mRNA and HSP70 mRNA expression more effectively than either monotherapy or NC control.(D) Consistent with mRNA results, chidamide and osimertinib combination treatment reduces SP1 protein levels more potently than single agents in both cell lines, as shown by Western blot.Immunoprecipitation (IP) of SP1 followed by acetylation detection (ACE-lysine)

reveals that chidamide treatment enhances SP1 acetylation in PC9/OR cells in a dose-dependent manner. IgG serves as a negative control.(F)Co-immunoprecipitation (Co-IP) shows that HDAC1 interacts with SP1 in PC9/OR cells, as detected by IB: SP1 and IB: HDAC1, suggesting that HDAC1 may regulate SP1 through deacetylation. (*, P < 0.05; **, P < 0.01, ***P < 0.001).



Knockdown of ARSD reduces the promoting effect of SP1 overexpression on osimertinib-resistant lung adenocarcinoma cells.

(A)In PC9/OR cells, ARSD knockdown reduces osimertinib IC50, while SP1 overexpression increases IC50. Co-expression of OE-SP1 with siARSD partially reverses the SP1-induced resistance. (B)In H1975/OR cells, similar results are observed: ARSD knockdown sensitizes cells to osimertinib, and siARSD partially reverses the resistance caused by SP1 overexpression.

conclusion

This study identified ARSD as a significantly upregulated resistance-related gene in an acquired osimertinib-resistant lung adenocarcinoma model. Chidamide was shown to regulate ARSD expression through acetylation and reverse the resistant phenotype. Mechanistically, chidamide inhibits HDAC1 activity, disrupts the interaction between HDAC1 and SP1, and reduces SP1-mediated transcriptional activation of ARSD, thereby downregulating ARSD expression and restoring sensitivity to osimertinib. Overall, the HDAC1-SP1-ARSD axis is an important mechanism of osimertinib resistance, and chidamide may serve as a potential combination therapeutic strategy.

