Deacetyl isovaltratum, an extract from *Valeriana*, induces G2/M phase arrest and apoptosis in gastric cancer cell lines

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Abstract

Background: *Valeriana* is a traditional Chinese herb, the root and rhizome of which has been used as a medicine against metrocarcinoma and cervical cancer. Deacetyl isovaltratum(DI) was isolated from *Valeriana* and its antitumor effect has been demonstrated in this work. The aim of this study is to investigate the antitumor effect of DI in gastric cancer cell line AGS and HGC-27 and the underlying mechanisms.

Methods: Microarray was used to analyze the differential expression profile before and after DI treatment. MTT assay and colony formation assay was used to detect the inhibitory effect of DI in various cancer cell lines. Flow cytometry was used to determine the cell cycle distribution and apoptotic cells. Tubulin polymerization assay was used to observe the in vitro microtubule polymerization process. Western blotting was utilized to determine the protein expression level.

Results: DI inhibited in vitro proliferation of various solid tumor cell lines, while showing prominent cytotoxicity to gastric cell lines. The IC_{50} of DI in gastric cancer AGS and HGC-27 cells was 5.78 ± 0.12 μM and 14.07 ± 0.55 μM respectively at 72 h treatment. Flow cytometry assay showed that DI induced G2/M phase arrest in AGS and HGC-27 cells. and promoted the polymerization of cell microtubule, thus inhibiting mitosis in gastric cancer cells. Up-regulation of the p-Chk1 protein and down-regulation of the cdc25c and cdc2 protein contributed to the cell cycle arrest caused by DI treatment.

Conclusions : DI could promote microtubule polymerization and caused G2/M phase arrest through regulating Chk1-cdc25c-cdc2 pathway. Extended exposure to DI further induced mitochondrion-dependent apoptosis in AGS and HGC-27 cells through the caspase-dependent pathway.

Keywords : Gastric cancer, DI, cell cycle, apoptosis

Results

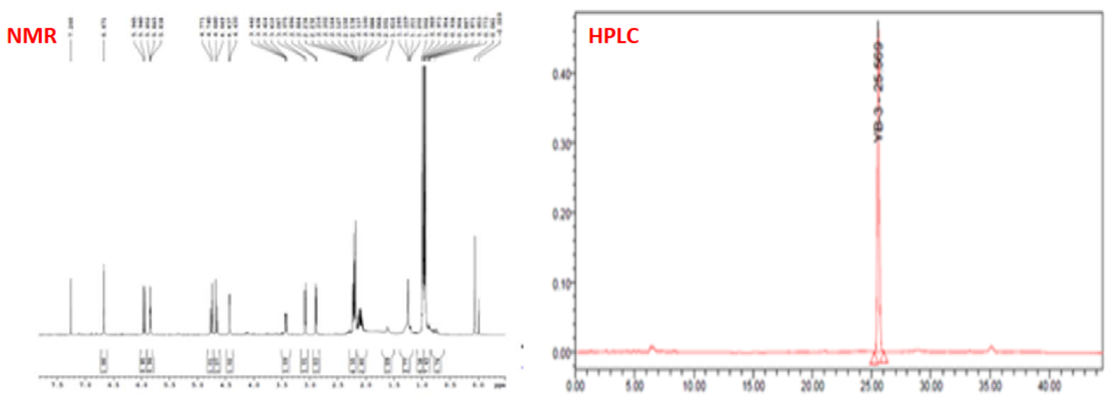

Figure 1. The characterization of DI. The structure of DI was verified by ^{1}H-NMR spectrum and the purity of isolated DI was demonstrated by HPLC analysis.

Cell Source	Cell lines	IC_{50} ± SD (μM)
Gastric Cancer	AGS	5.78 ± 0.12
Non-Small Cell Lung Cancer	H1650	8.34 ± 0.12
Esophageal Cancer	TE-3	16.16 ± 0.55
Breast Cancer	MCF-7	37.54 ± 0.83
Prostate Cancer	PC-3	12.46 ± 0.32
Pancreatic Cancer	CFPAC-1	6.61 ± 0.42
Glioma	U251	16.79 ± 0.85
Colorectal Cancer	HCT-116	10.10 ± 0.34

Figure 2. The in vitro IC50 of DI against cancer cell lines. Cells were treated with DI for 48 h before MTT assay.

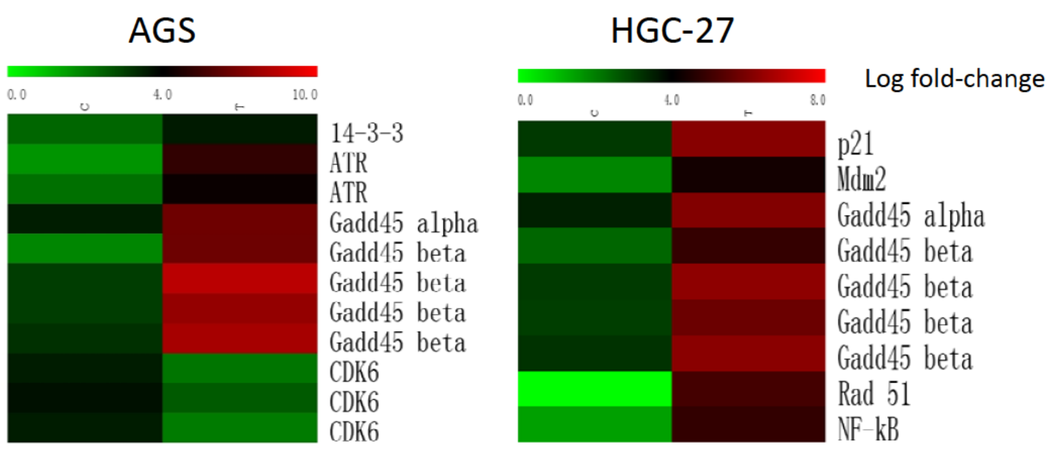

Figure 3. The log fold-change heatmap of cell cycle related gene expression before (C) and after (T) treatment with 12 μM and 30 μM of DI in AGS and HGC-27 cells respectively.

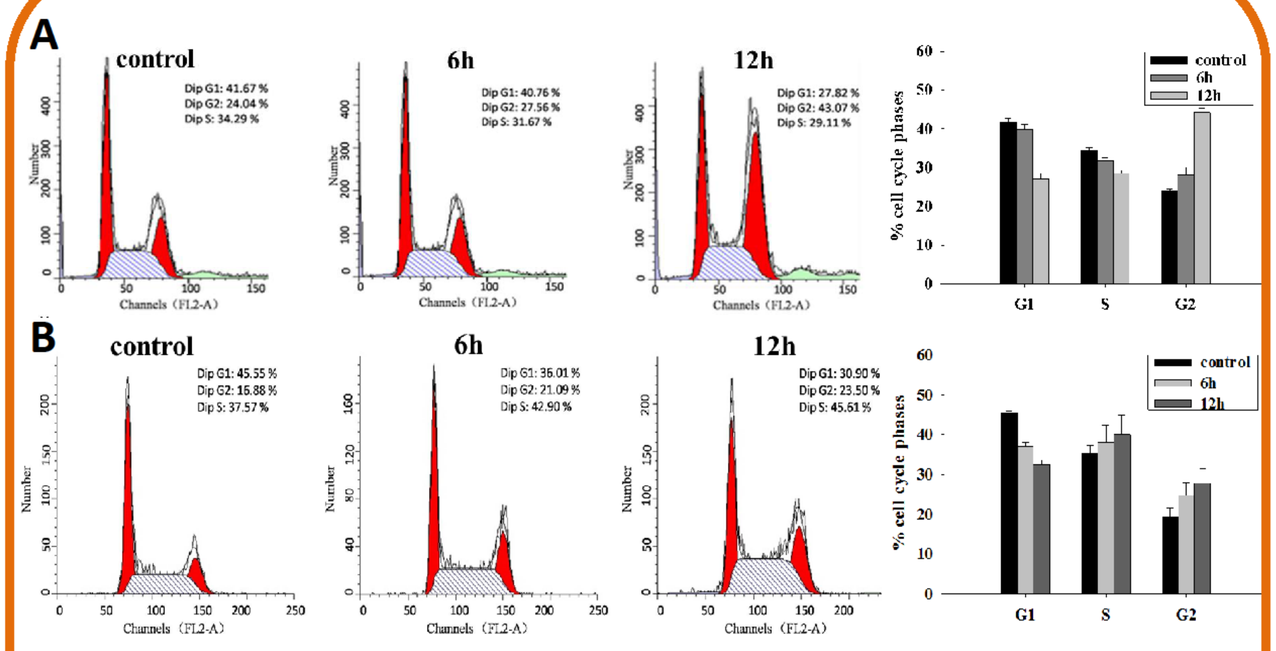

Figure 4. DI induced G2/M phase arrest in AGS (A) and HGC-27 (B) cells. Cells were treated with 12 μM (AGS) or 30 μM (HGC-27) of DI for 24 h before harvesting and fixation with 70 % ethanol, followed by PI stain and flow cytometry analysis.

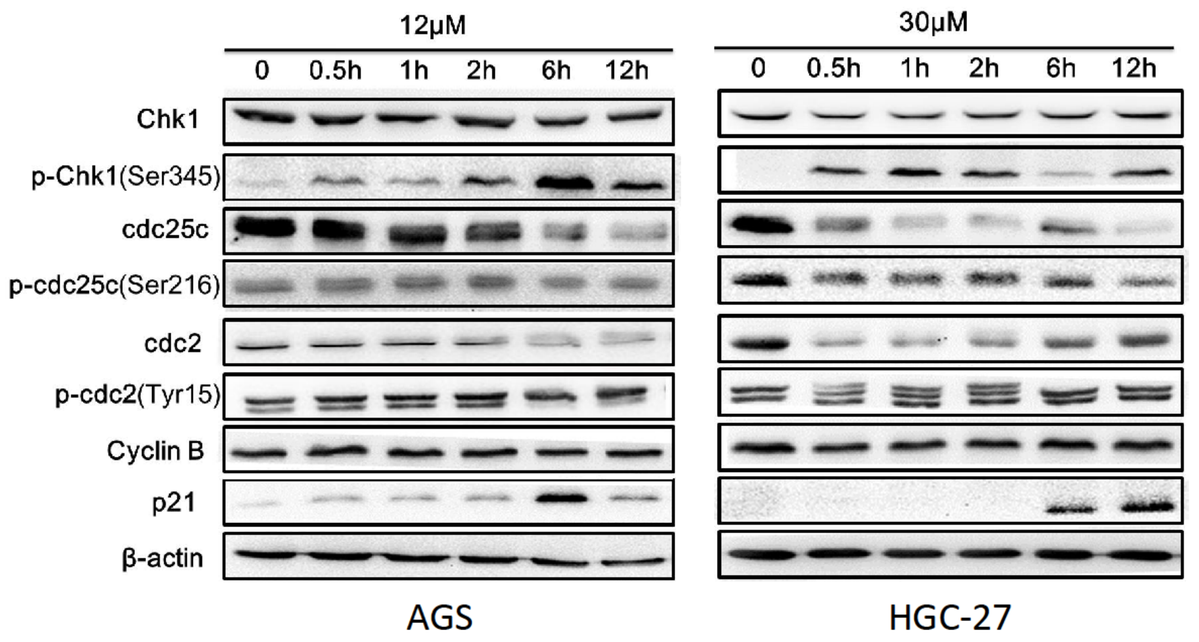

Figure 5. The effect of DI on cell cycle related protein expression in AGS and HGC-27 cells. Cells were treated with 12 μM (AGS) or 30 μM (HGC-27) of DI for 48 h before western blotting assay.

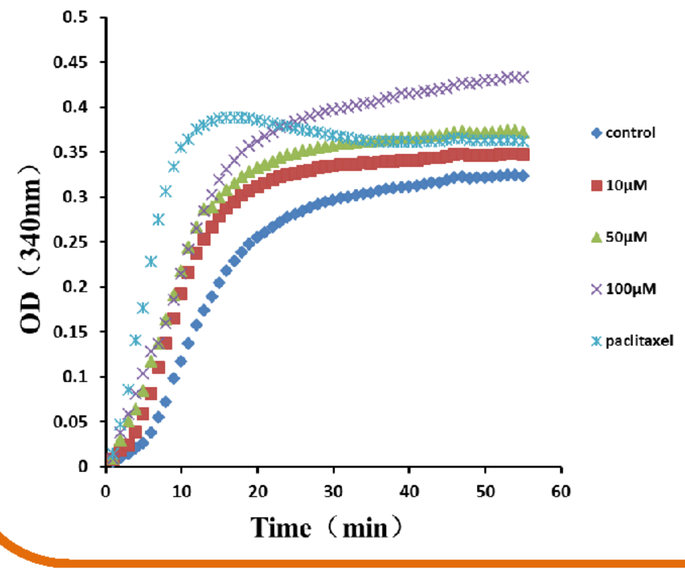

Figure 6. DI expedited in vitro tubulin polymerization process. The tubulin polymerization assay was performed according to the provider's instruction. Untreated tubulin solution was used as negative control and paclitaxel was used as positive control.

Conclusion

DI is a pure compound isolated from *Valeriana,* and the structure of DI has been well demonstrated. In this study, DI showed in vitro anti-cancer activity in gastric cell lines through the induction of G2/M phase arrest. Further study showed that DI promoted tubulin polymerization, which contributed to its G2/M phase arrest activity. Next, we will dedicate to explore the potential molecular target of DI, explicating the anti-cancer mechanism of this newly extracted natural product.

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WCGC 2015
18--P
Basic Gastric Cancer
Bo Zhang
DOI: 10.3252/pso.eu.17wcgc.2015
Poster presented at: 17th World Congress on Gastrointestinal Cancer, 1–4 JULY 2015, BARCELONA, SPAIN