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Apicidin as Potential Antiproliferative Agent

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Apicidin [cyclo(*N*-*O*-methyl-L-tryptophanyl-L-isoleucinyl-D-pipecolinyl-L-2-amino-8-oxo decanoyl)] is a fungal metabolite shown to exhibit antiparasitic activity by the inhibition of histone deacetylase (HDAC). In this study, we evaluated apicidin as a potential antiproliferative agent. Apicidin showed a broad spectrum of antiproliferative activity against various cancer cell lines even though with differential sensitivity. The antiproliferative activity on HeLa cells by apicidin was accompanied by morphological changes, cell cycle arrest at G_1 phase, and accumulation of hyperacetylated histone H4 *in vivo* as well as inhibition of partially purified HDAC *in vitro*.

Table 1 Growth inhibitory concentrations of apicidin on various cell lines

Exponentially growing mouse and human cell lines were treated with various concentrations of apicidin for 48 h, and the viable cell numbers were determined by SRB assay, as described under materials and methods. The results are mean of triplicate from three separate experiments.

Cell line	IC_{50}, *μg/ml*
CCD-18Co	2.36
HeLa	0.51
v-ras-transformed NIH3T3	0.18
Colon 3.1-M26	0.17
MG63	1.88
MCF7	1.17
HBL-100	0.57
AGS	0.13
A2058	0.55
ZR-75-1	1.17

In addition, apicidin induced selective changes in expressions of $p21^{WAF1/Cip1}$ and gelsolin, which control the cell cycle and cell morphology, respectively. Consistent with increased induction of $p21^{WAF1/Cip1}$, phosphorylation of Rb protein was markedly decreased, indicating the inhibition of cyclin-dependent kinases (CDKs), which became bound to $p21^{WAF1/Cip1}$. The effects of apicidin on cell morphology, expression of gelsolin, and HDAC1 activity *in vivo* and *in vitro* appeared to be irreversible, because withdrawal of apicidin did not affect those effects, whereas the induction of $p21^{WAF1/Cip1}$ by apicidin was reversible. Taken together, the results suggest that induction of histone hyperacetylation by apicidin is responsible for the antiproliferative activity through selective induction of genes, which play important roles in the cell cycle and cell morphology.

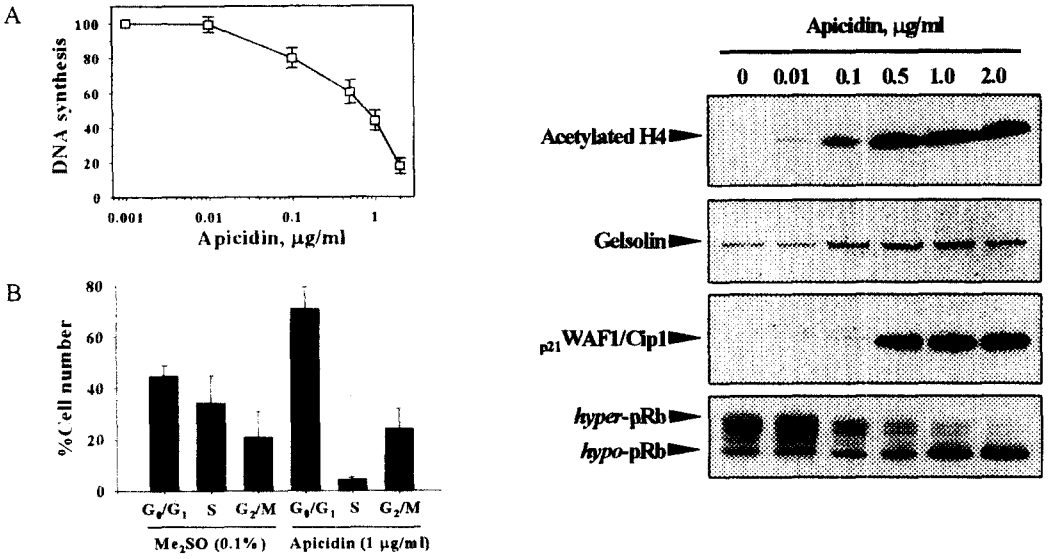

Figure. 2. ***left,*** Effect of apicidin on the cell cycle progression of HeLa cells. (A) HeLa cells were treated with various concentrations of apicidin for 24 h, and then DNA synthesis was determined by incubation with 5 x 10^4 cpm [^{3}H]thymidine/ml (B) After incubation with 1 μg/ml apicidin for 24 h, HeLa cells were collected and their isolated nuclei were analyzed by flow cytometry. ***right,*** Lysates (30 μg) or histones (for acetylated H4 analysis) of the HeLa cells exposed to 0.01, 0.1, 0.5, 1, or 2 μg/ml apicidin (lanes 2, 3, 4, 5, and 6, respectively) or 0.1% Me2SO (lane 1) were subjected to immunoblotting.