S-5 [14:30~15:00]

Apicidin, a Histone Deacetylase Inhibitor, as a Potential Anticancer Agents

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Apicidin [cyclo(N-O-methyl-L-tryptophanyl-L-isoleucinyl-D-pipecolinyl-L-2-amino-8-oxodecanoyl)] 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 (Table 1). 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* (Fig. 1). 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 $p21^{WAF1/Cip1}$ (Fig. 2). In further study, we investigated the mechanism of apicidin-induced transcriptional activation of $p21^{WAF1/Cip1}$ and the possible involvement of protein kinase signaling pathway in the $p21^{WAF1/Cip1}$ gene expression. Apicidin induced $p21^{WAF1/Cip1}$ gene expression independent of the *de novo* protein synthesis through Sp1-3 site located at –82 and –77 relative to the transcription start site of $p21^{WAF1/Cip1}$. Moreover, either pretreatment with the PKC inhibitor, calphostin C or overexpression of dominant negative form of PKCε, significantly attenuated the apicidin-induced activation of $p21^{WAF1/Cip1}$ promoter, expression of $p21^{WAF1/Cip1}$ mRNA and protein, and translocation of PKCε from cytosolic to particulate fraction (Fig. 3). Taken together, these results suggest that PKC signal transduction pathway is involved in the apicidin-induced transcriptional activation of the $p21^{WAF1/Cip1}$ gene expression via Sp1 sites.

In addition, we evaluated the apoptotic potential of apicidin in human acute promyeolcytic leukemia cells HL60 and investigated the mechanism of apicidin-induced apoptosis by analyzing the signaling pathway of apoptosis. Apicidin induced apoptosis via a mitochondrial/cytochrome *c*-dependent pathway, which require de novo protein synthesis of Fas/Fas ligand, resulting in cytochrome *c* release from the mitochondria to the cytosol and subsequent activation of caspase-9 and caspase-3 (Fig. 4).

Taken together, these studies provide insight into molecular mechanisms of apicidin-induced cell cycle arrest and apoptosis of tumor cells and suggest that apicidin might be a new class of potential therapeutic agent for cancer treatment.

Table 1. Growth inhibitory concentrations of apicidin on various cell lines

Cell line	IC50, 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

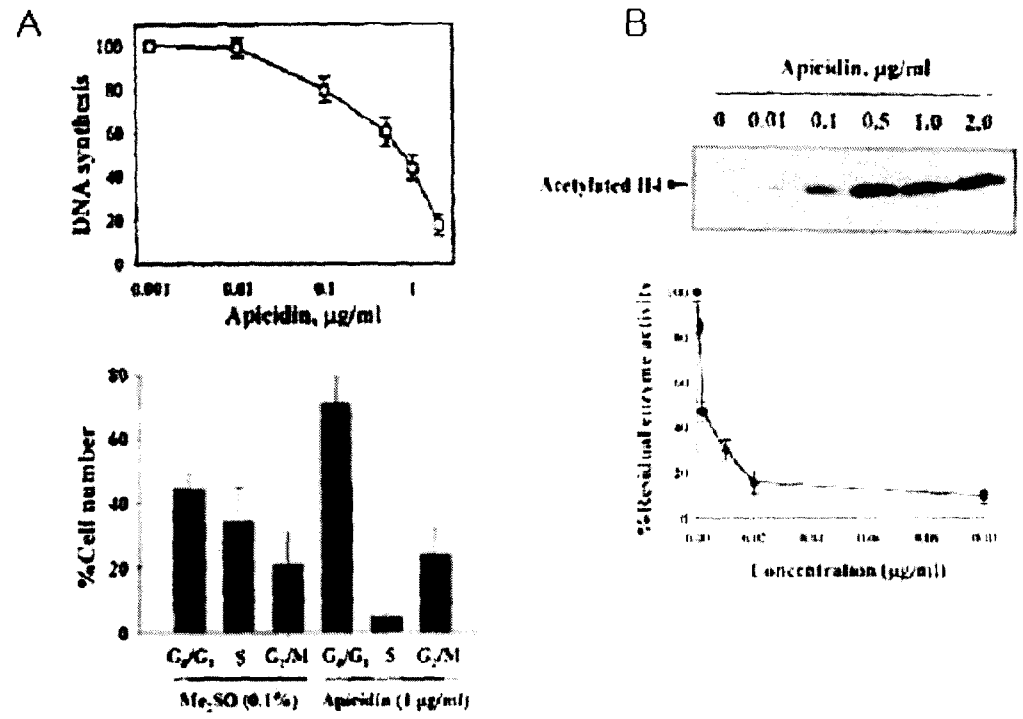

Fig. 1. Effect of apcidin on cell cycle progression and HDAC activity in HeLa cells

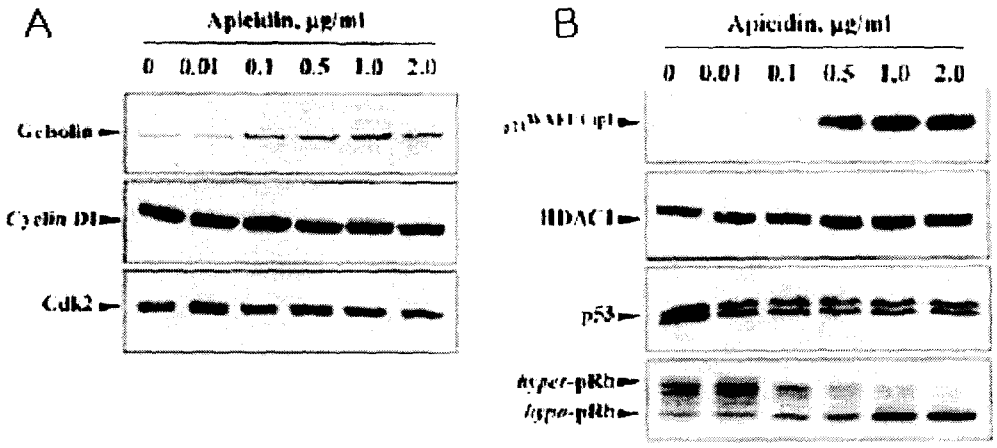

Fig. 2. Effect of apcidin on the expression of endogenous genes in HeLa cells.

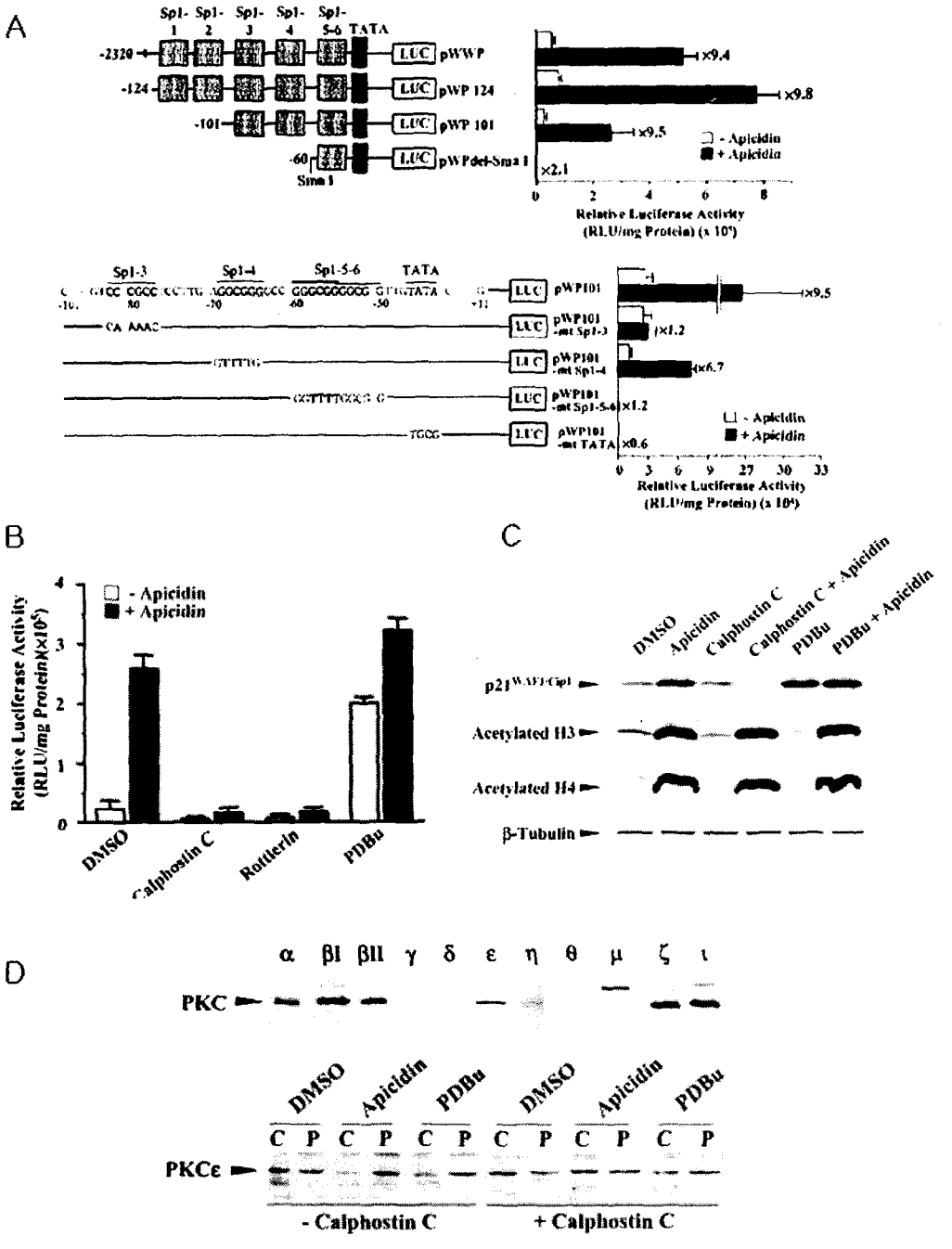

Fig.3. PKC signal transduction pathway is involved in the apicidin-induced transcriptional activation of the p21 $^{WAF1/Cip1}$ gene expression via Sp1 site

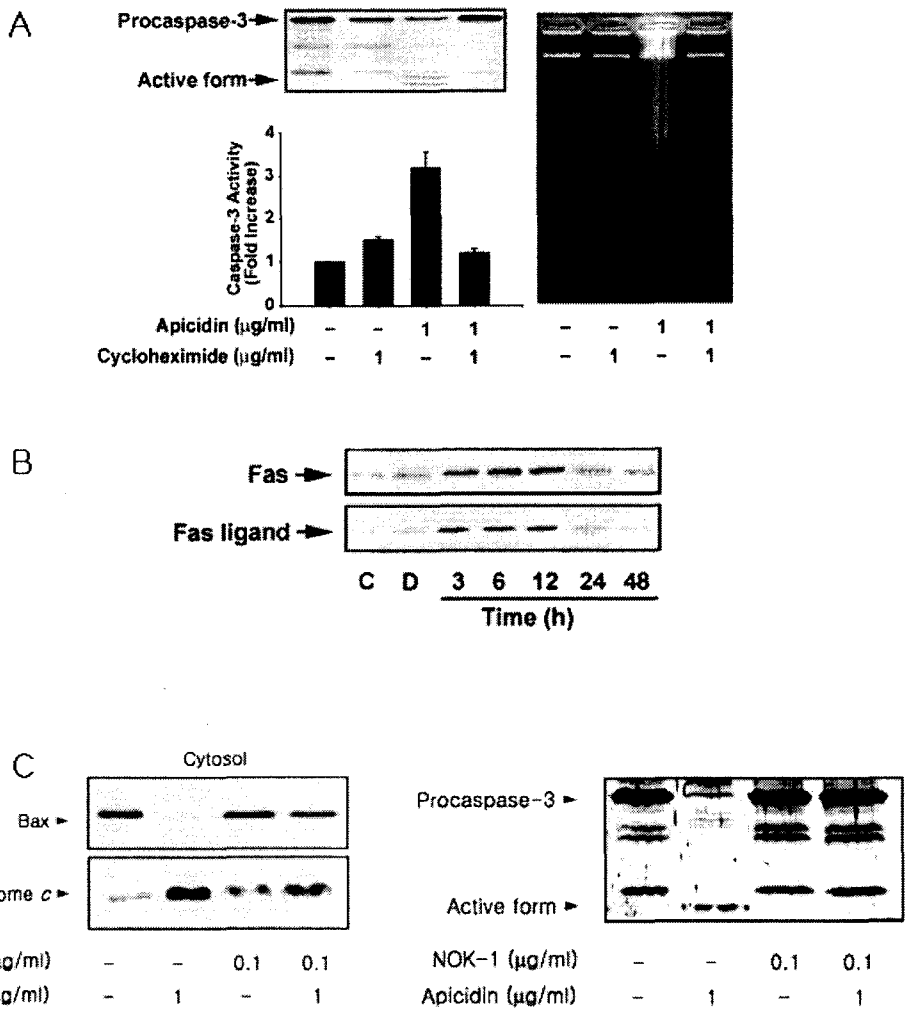

Fig. 4. Apcidin induced apoptosis via a Fas/Fas liganed expression