OP-05

WJ25591, A HDAC Inhibitor, Displays Anti-cancer Activity Through Cell Cycle Arrest and Apoptosis in PC-3 Cells

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WJ25591 was developed from the hybridization of suberoylanilide hydroxamic acid (SAHA) and panobinostat (LBH-589), two pan histone deacetylase (HDAC) inhibitors currently used in clinical studies. The fluorescence-based HDAC activity assay showed that WJ25591 inhibited the activity of HDAC-1, -2 and -6 with IC_{50} values of 312, 580 and 578 nM, respectively. The sulforhodamine B assay demonstrated that WJ25591 inhibited the proliferation of human androgen independent prostate cancer cell line PC-3 with an IC_{50} of 0.45 μM. Accordingly, the intracellular signaling pathways were elucidated. WJ25591 induced a time-related arrest of cell cycle at G2/M- and G1-phase and a subsequent increase of sub-G1 population (apoptosis) using flow cytometric analysis of propidium iodide staining. The Western blot showed that WJ25591 resulted in a strong induction of p21 expression, a cyclin-dependent kinase (CDK) inhibitor. In contrast, the expressions of some key cell cycle regulatory proteins including cyclin D1, cyclin A, cyclin B1 and survivin were down-regulated. Besides, WJ25591 induced the activation of caspase-8, -9 and -3 and the cleavage of PARP-1 suggesting the involvement of caspase-dependent apoptotic pathways. The activation of extrinsic apoptotic pathway was further identified by the evidence that WJ25591 triggered FADD translocation from cytosol to cell membrane by confocal microscopic examination. The anticancer effect of combination use of WJ25591 and several chemotherapeutic agents was also determined. The data demonstrated that WJ25591 combined with MG-132, a proteasome inhibitor, profoundly potentiated apoptosis in PC-3 cells. The study of several intracellular signals, including the increased expression levels of acetyl-histone H3, GRP-78, p-H2AX and the cleaved caspase-3, caspase-8 and PARP-1, confirmed the synergistic effect of combination treatment. In conclusion, the data suggest that WJ25591 inhibits HDAC activity, leading to an arrest of the cell cycle and subsequent caspase-dependent apoptosis. WJ25591 also displays a synergistic anticancer activity in combination with proteasome inhibitor.