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CELL CYCLE ARREST AND INDUCTION OF APOPTOSIS BY NOVEL CDK INHIBITOR IS ASSOCIATED WITH $p16I^{NK4A}$ UP-REGULATION IN HUMAN PROMYELOCYTIC LEUKEMIA CELLS

Bu-Young Choi[1,2], Min-Kyoung Kim[2], Hak-Yup Kim[1], Youl-Hee Cho[2], and Chul-Hoon Lee[2]

[1]Department of Molecular Biology and Pharmacology, C&C Research Laboratories, Kyunggi-Do
[2]Department of Medical Genetics, College of Medicine, Hanyang University, Seoul, Korea

Purpose: MCS-5A, novel Cdk inhibitor, has been reported that it has exerted cell cycle arrest action and apoptotic effect to the human promyelocytic leukemias cell. The purpose of this study is to verify these effects of MCS-5A on human promyelocytic leukemia (HL-60) cells and to clarify the action of mechanism on MCS-5A-inducing apoptosis.

Methods: Untreated and treated HL-60 cells were evaluated for antiproliferative effect and apoptosis using cell viability test, protein kinas assay, DNA fragmentation, Flow cytometry assay, and light and electromicroscopic examination. To clarify the action of mechanism, we also performed co-immunoprecipitation, immunoblot assay for cell cycle and apoptosis proteins and constracted of pcDNA3.1-$p16I^{NK4A}$. To determine the possible of apoptosis to pcDNA-p16 mediated cytotoxicity, the TUNEL(TdT mediated dUTP-biotin nick end labeling)assay were performed by flow cytometry on A549 cell, human lung cancer cell, which has homozygous deletion of p16.

Results: We investigated the involvement of cell cycle regulatory events during MCS-5A mediated apoptosis in HL-60 cells. The treatment of HL-60 cells with MCS-5A (3uM, 12hrs) resulted in inhibition of the phosphorylation of Rb protein, a critical step for G1/S transition. The kinase activities of G1/S and G2 cyclin-dependent kinases (Cdk4, Cdc2) were inhibited in HL-60 cells treated with MCS-5A(IC_{50} values of 8.8 and 9.6 μM, respectively). Furthermore, MCS-5A increases the level of Cdk inhibitor p16 and had no effect on those of p21. MCS-5A promoted binding of p16 to Cdk4. The induction of apoptosis by MCS-5A is associated with p16 up-regulation. Transient transfection of

A549 cell(p16 negative) with pcDNA-p16 resulted in a rightward shift of the mean fluorescene intensity when compared to the baseline fluorescence following tansfection with pcDNA vector. MCS-5A can induce apoptosis through different pathway of caspase activation with caspase-8 and caspase-9 playing a pivotal role. Caspase-8 can also activate the pro-apoptotic Bcl-2 family member Bid through proteolytic cleavage. The activation of caspase-9 in MCS-5A treated HL-60 cells is likely to occur via the caspase-8-Bid-mitochondria pathway which leads to cytochrome c release, followed by cleavage of caspase-9. MCS-5A exerted antiproliferation and the growth inhibition of HL-60 through the induction of apoptosis which is mediated p16 and via Bcl-2 insensitive activation of caspase-3.

Conclusions: These results indicate that MCS-5A exerts cell cycle arrest and apoptosis inducing activity in HL-60 cells and might have a potent activity as a new concept anticancer agent in human leukemias and that p16 is capable of mediating apoptosis in human cancer cells