SUMMARY OF Ph.D. DISSERTATION

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Title

Studies on the inhibitory effects of HIV-1 Vpr and terpendole E on cell division

Abstract

To elucidate the inhibitory mechanisms of the inhibitors or proteins, which prevent cell cycle progression, will provide useful information for the cell cycle study. We investigated the inhibitory mechanisms about HIV-1 Vpr and terpendole E.

(1) Growth inhibitory effects of HIV-1 Vpr

HIV-1Vpr (viral protein R) that increases HIV-1 replication in nondividing macrophages localizes to the nucleus and blocks cell cycle at the G2 checkpoint in mammalian cell. Vpr also inhibits the growth of yeast. In this study, we used the budding yeast, *S. cerevisiae* to elucidate the precise mechanisms of the growth inhibition caused by Vpr. We found that Vpr can arrest the growth of yeast deficient in proteins on DNA damage or replication checkpoint pathway and we concluded that Vpr arrests cell growth independently from the checkpoint pathway in *S. cerevisiae*. Using point mutated Vpr, we also identified growth inhibition occurred independently from the localization of Vpr in yeast. Furthermore, several mutations canceled growth inhibitory activity and/or changed its intracellular localization in both yeast and mammalian cells, suggesting the importance of these residues for the phenotype.

(2) The inhibitory effects of Terpendole E on cell division

Terpendole E that had been known as an ACAT (acyl-CoA: cholesterol O-acyltransferase) inhibitor, was rediscovered from microbial metabolites as an M phase inhibitor. Interestingly, terpendole E did not show any effects on microtubule integrity. Terpendole E induced the formation of a monoastral spindle instead of a normal bipolar spindle in M-phase. It is known that the mitotic kinesin Eg5 functions in the establishment of bipolar spindle and its inhibition results in the monoastral spindle. To examine the possibility that the molecular target of terpendole E might be Eg5, we measured the motility activity and ATPase activity of Eg5 *in vitro* in the presence of terpendole E. Terpendole E inhibited both motility and ATPase activity of Eg5, but it did not inhibit those of the other kinesin (KHC). Furthermore other terpendoles, which are also known as ACAT inhibitors, did not inhibit motility or ATPase activity of both Eg5 and KHC. These results suggested that terpendole E is the first natural and specific inhibitor for mitotic kinesin, Eg5.

As indicated above, it is very important in the cell cycle study to elucidate the inhibitory mechanisms of the specific inhibitors or the protein that inhibit cell cycle progressions.