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GPScreen™을 이용한 천연 항암물질인 plumbagin의 약물 작용점 연구; 분열 효모인 *S. pombe* 유전체 이종 결손 변이 라이브러리에서의 약물에 의한 haploinsufficiency를 이용한 약물 작용점 규명을 위한 혁신 기술.

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Drug Target Identification of a natural anticancer agent plumbagin using GPScreen™; An innovative Technology for Drug Target Discovery using Drug-induced haploinsufficiency in *S. pombe* Genome-wide Heterozygous Deletion Mutant Library

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실험목적 (Objectives)

Drug-induced haploinsufficiency(DIH) in yeast is a valuable tool for identification of drug target and its mode-of-action. In this study, we have tried to identify its detailed molecular target of a natural anticancer agent plumbagin using an innovative DIH-based drug target screening system GPScreen™.

재료 및 방법 (Materials and Methods)

○ 실험재료

- Bioneer's unique *S. pombe* Genome-wide deletion mutant library
- Plumbagin was purchased from Sigma.

○ 실험방법

S. pombe wild-type or mutant cells were cultured in YES media and plumbagin was treated to cells in 96-well plates and incubated for 14 h and DIH was measured using GPScreen™ at 600 nm in microplate reader in the presence of plumbagin.

실험결과 (Results)

Plumbagin induced a potent anti-proliferative activity and the cell elongation and septum formation. Interestingly, DIH was found in *its3*-deleted mutant, suggesting that *its3* might be a molecular target of the agent. In mode of actions, plumbagin induced a significant increase of ROS and pretreatment of an ROS scavenger NAC protected the growth inhibition by plumbagin, suggesting that ROS might be a critical mediator for the cytotoxicity. Plumbagin also induced DIH in TOR2-deleted mutant. Plumbagin in decreased PI-5 kinase-1B mRNA in human cancer cells, which is a human ortholog of *its3*. These results show that PI-5 kinase-1B is another molecular target of plumbagin and GPScreen™ drug target screening system is a valuable tool for identification of both the drug target and mode-of-action of drug candidates.

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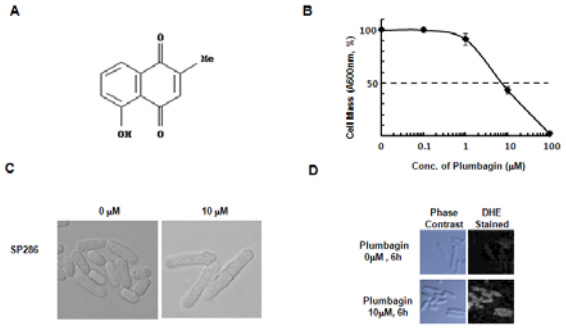
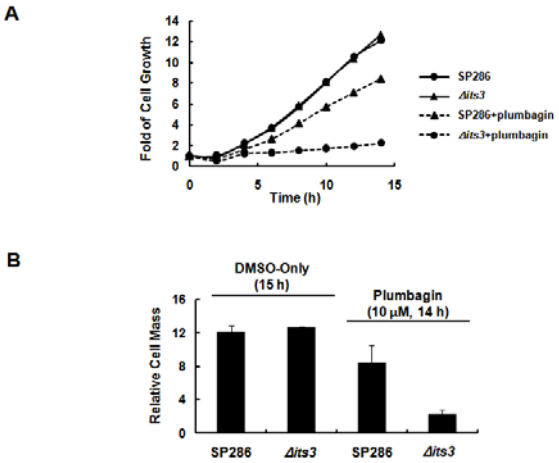
* 시험성적

I. Plumbagin induced a potent growth inhibition and ROS in wild type *S. pombe*.

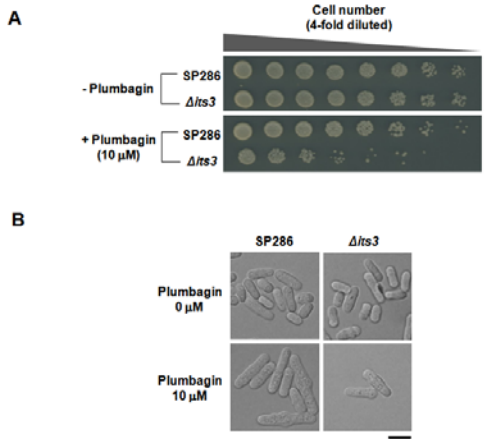


A. Structure of Plumbagin,
B. Growth-inhibitory activity(GI50; 10uM)
C. Cell elongation and septum Formation by Plumbagin
D. ROS induction by Plumbagin

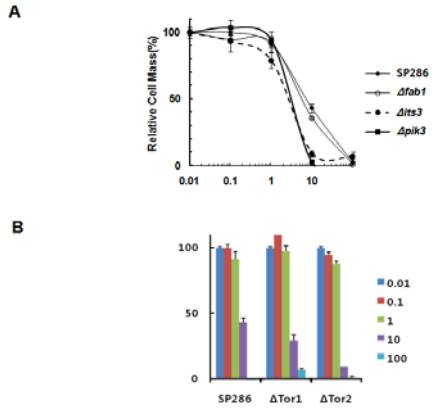
II. The growth inhibition by plumbagin was dramatically increased in *its3*-deleted mutant.



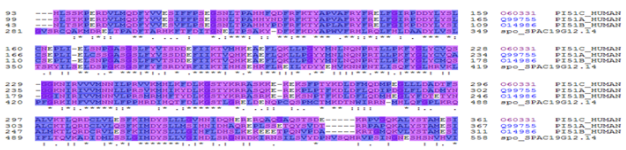
III. The growth inhibition by plumbagin was dramatically increased in *its3*-deleted mutant.



IV. Drug-induced Haploinsufficiency was also occurred in PI-3 kinase & *tor2*-deleted mutants.



V. Human orthologs of *S. pombe its3*; PI-5 kinases



VI. Down-regulation of PI5K-1B by plumbagin in Human Breast cancer MCF-7 cells.