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Dihydrotanshinone I is an Inhibitor of Farnesyl-Protein Transferase

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Abstract

An inhibitor of farnesyl-protein transferase is known to be a good candidate for antitumor agent that block the oncogenic activity of Ras protein. We recently isolated and characterized dihydrotanshinone I from Salvia miltiorrhiza Bunge (Danshen), an oriental herb, which has an inhibitory activity of topoisomerase I to some cancer cell lines. In order to examine the molecular mechanism of dihydrotanshinone I, we studied the farnesyl-protein transferase activity by dihydrotanshinone I. As a result, we found that dihydrotanshinone I showed inhibitory effect on farnesyl-protein transferase with IC_{50} value of 15 ug/ml. This result suggest that dihydrotanshinone I may be an useful anticancer agent with the inhibitory activity of farnesyl-protein transferase.

Key words : farnesyl-protein transferase, Ras, dihydrotanshinone I, cryptotanshinone, anticancer agents

The ras oncogenes and the proteins(Ras) encoded by these genes have been the subjects of intense investigation for nearly two decades. Oncogenic activation of Ras protein requires post-translational modifications and proper membrane association to transform cells(1). Localization of Ras protein in membrane is dependent on three posttranslational modifications : farnesylation, proteolysis, and methyl esterification(2). Inhibition of any of these three steps would interfere membrane localization and transforming activity of ras oncogene(3). Several reports have, therefore, focused on developing inhibitors of farnesyl-protein transferase(FPTase) as potential antitumor agents(4). Of them, chaetomellic acids A and B(5,6), fusidienol(7), zaragozic acid A(8), zaragozic acids B, C, D, and D2(9), manumycin(10), pepticinnamins(2), 10'-demethoxystreptonigrin(11), and gliotoxins(12) inhibited farnesyl-protein transferase in vivo. In the course of search for effective agents for farnesyl-protein transferase, we first found an antitumor agent dihydrotanshinone I(Fig. 1) isolated from Salvia miltiorrhiza Bunge(Danshen). In order to examine whether the agent inhibit the farnesyl-protein transferase or not, we measured the activity of FPTase carried out by Brown et al.(13) with a slight modification using[^{3}H〉-farnesyl pyrophosphate(FPP) as a substrate. Rat brain FPTase was purified by the method reported by Reiss et al.(11). The reaction mixture was contained as follows : 10 ul of assay buffer(50 mM Tris-HCl, pH 7.5,

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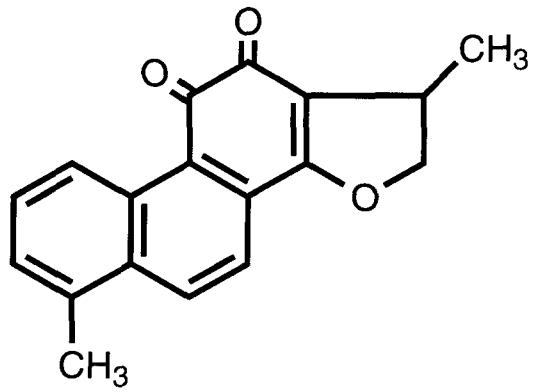
25 mM $MgCl_2$, 2 mM KCl, 5 mM Na_2HPO_4, and 0.01 % Triton X-100), 10 ul of purified dihydrotanshinone I(0.1, 1, 10, and 100 ug/ml), 20 ul of pre-diluted [^{3}] FPP, 20 ul of biotin-lamin B peptide, and 40 ul of pre-diluted FPTase. The mixture was incubated at 37℃ for 30 min and the reaction was stoped by adding 150 ul of the stop/beads reagent. The radioactivity was measured using a scintillation counter(Packard). As shown in Fig.2, dihydrotanshinone I had inhibitory effect on FP-Tase with IC_{50} value of 15 ug/ml, but cryptotanshinone had no significant effects in spite of similar structure with dihydrotanshinone I.(data not shown).



Dihydrotanshinone I

Fig. l engends;Fig. 1. Structure of dihydrotanshinone I.

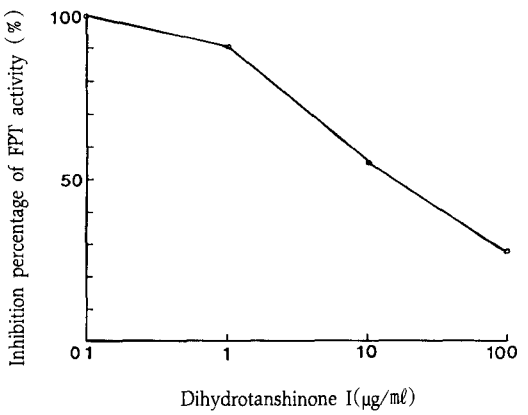


Fig. 2. Effect of dihydrotanshinone I on the inhibition of farnesyl-protein transferase activity.

In the recent study, we found that dihydrotanshinone I is also a potent inhibitor of DNA topoisomerase I, and inducer of apoptosis(unpublished data). Accordingly, we can not rule out whether the damage of cells by dihydrotanshinone I results from direct inhibition of FPTase activity.

In summary, we found that dihydrotanshinone I is an inhibitor of farnesyl-protein transferase. Because little studies on inhibitors from plant-origin studied, although it is well investigated that various peptidometic inhibitors of FPTase suppress Ras-mediated cell transformation by preventing farnesylation of the Ras oncoprotein (14-16), we are anticipating that dihydrotanshinone I may be a good candidate for anticancer agents from oriental herb origin.

References

1. Barbacid, M. : Ras genes. Annu. Rev. Biochem., **56**, 779(1987).
2. Omura, S., Van Der Pyl, D., Inokoshi, J., Takahashi, Y., and Takeshima, H. : Pepticinnamins, new farnesyl-protein transferase inhibitors produced by an actinomycete. *J. Antibiot.,* **46**, 222(1993).
3. Schafer, W. R., Kim, R., Sterne, R., Thorner, J., Kim, S. H., and Rine, J. : Genetic and pharmacological suppression of oncogenic mutations in Ras genes of yeast and human. *Science,* **245**, 379(1989).
4. Tamanoi, F. : Inhibitors of Ras farnesyl transferase. *Trends Biochem. Sci.,* **8**, 349(1993).
5. Lingham, R. B., Silverman, K. C., Bills, G. F., Cascales, C., Sanchez, M., Jenkins, R. G., Gartner, S. E., Martin, I., Diez, M. T., Pelaez, F., Mochales, S., Kong, Y. L., Burg, R. W., Meinz, M. S., Huang, L., Nallin-Omsteadb, M., Mosser, S. D. Schaber, M. D., Omer, C. A., Pomplinao, D. L., Gibbs, J. B., and Singh, S. B. : Chaetomella acutiseta produces chaetomellic acids A and B which are reversible inhibitors of farnesyl-protein transferase. Appl. Microbiol. Biotechnol., **40**, 370(1993).
6. Singh, S. B., Zink, D. L., Liesch, J. M., Goetz, M. A.,

Jenkins, R. G., Nallin-Omstead, M., Silverman, K. C., Bills, G. F., Mosley, R. T., Gibbs, J. B., Albers-Schonberg, G., and Lingham, P. R. : Isolation and structures of chaetomellic acids A and B from Chaetomella acutiseta : farnesyl pyrophosphate mimics inhibitors of Ras farnesyl-protein transferase. *Tetrahedron,* **49**, 5917(1993).

7. Singh, S. B., Jones, E. T., Goetz, M. A., Bills, G. F., Nallin-Omstead, M., Jenkins, R. G., Lingham, P. R., Silverman, K. C., and Gibbs, J. B. : Fusidienol : a novel inhibitor of Ras farnesyl-protein transferase from Fusidium griseum. *Tetrahedron Letters,* **27**, 4693(1994).
8. Gibbs, J. B., Pompliano, D. L., Mosser, S. D., Rands, E., Lingham, R. B., Singh, S. B., Scolnick, E. M., Kohl, N. E., and Oliff, A. : Selective inhibition of farnesyl-protein transferase blocks Ras processing in vivo. *J. Biol. Chem.,* **268**, 7617(1993).
9. Dufresne, C., Wilson, K. E., Singh, S. B., Zink, D. L., Bergstrom, J. D., Rew, D., Polishook, J. D., Meinz, M. S., Huang, L., Silverman, K. C., Lingham, R. B., Mojena, M., Cascales, C., Pelaez, F., and Gibbs, J. B. : Zaragozic acid D and D2 : potent inhibitors of squalene synthase and of Ras farnesyl protein transferase. *J. Nat. Prod.* **56**, 1923(1993).
10. Hara, M., Akasaka, K., Akinaga, S., Okabe, M., Nakano, H., Gomez, R., Wood, D., Uh, M., and Tamanoi, F. : Identification of Ras farnesyl transferase inhibitors by microbial screening. *Proc. Natl. Acad. Sci. U.S.A.,* **90**, 2281(1993).
11. Liu, W. C., Barbacid, M., Bulgar, M., Clark, L. M., Crosswell, A. R., Dean, L., Doyle, T. W., Fernandes, P. B., Huang, S., Manne, V., Pirnik, D. M., Well, J. S., and Meyers, E. : 10'-Desmethoxystreptonigrin, a novel analog of streptonigrin. *J. Antibiot.,* **45**, 454(1992).
12. Van Der Pyl, D., Inokoshi, J., Shioma, K., Yang, H., Takeshima, H., and Omura, S. : Inhibition of farnesyl-protein transferase by gliotoxin and acetylgliotoxin. *J. Antibiot.,* **45**, 1802(1992).
13. Brown, M. S., Goldstein, J. L., Paris, K. J., and Burnier, M. S. : Tetrapeptide inhibitors of protein farnesyltransferase : amino-terminal substitution in phenylalanine-containing tetrapeptides restores farnesylation. *Proc. Natl. Acad. Sci. U.S.A.,* **89**, 8313(1992).
14. Kauffmann, R. C., Qian, Y., Vogt, A., Sebti, S. M., Hamilton, A. D., and Carthew, R. W. : Activated Drosophila Ras 1 is selectively suppressed by isoprenyl transferase inhibitors. *Proc. Natl. Acad. Sci. U.S.A.,* **92**, 10919(1995).
15. Kohl, N. E., Mosser, S. D., deSolms, S. J., Giuliani, E. A., Pompliano, D. L., Graham, S. L., Smith, R. L., Scolnick, E. M., Oliff, A., and Gibbs, J. B. : Selective inhibition of ras-dependent transformation by a farnesyl transferase inhibitor. *Science,* **260**, 1934(1993).
16. Wallace, A., Koblan, K. S., Hamilton, K., Marquis, O. D., Miller, P. J., Mosser, S. D., Omer, C. A., Schaber, M. D., Cortese, R., Oliff, A., Gibbs, J. B., and Pessi, A. : Selection of potent inhibitors of farnesyl-protein transferase from a synthetic tetrapeptide combinatoriallibrary. *J. Biol. Chem.,* **271**, 31306(1996).

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초록 : Farnesyl-Protein Transferase의 저해제 Dihydrotanshinone I.

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Farnesyl-protein transferase의 저해제는 Ras단백질의 발암활성을 차잔하는 항암제의 후보로서 알려져 있다. 우리는 최근에 topoisomerase I에 대하여 저해활성을 갖는 dihydrotanshinone I을 약용식물인 *Salvia miltiorrhiza* Bunge(Danshen)으로부터 분리하였다. Dihydrotanshinone I의 작용기작의 해석을 위한 시도에서 farnesyl-protein transferase에 대한 저해능(IC_{50}치=15ug/ml)을 관찰하였으며, 이것은 유용한 항암제로서의 가능성을 제시한 결과로 본다.