A real-time biosensor system *in vivo* using luciferase as an intracellular reporter for the functional analysis of anti-cancer compounds based on Hsp70 inhibition

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Abstract

Introduction: Defective apoptosis (programmed cell death) is a major contributor to the process of cancer progression and metastasis .Over the last decade, new drug compounds were designed to enhance apoptosis in the tumor cells. Hsp70 chaperone is an anti-apoptotic protein and its inhibitors serve as suitable compounds in cancer treatment strategies. A number of different techniques such as x-ray crystallography are available for studying inhibitory effects of apoptosis-inducing compounds on Hsp70 activity. These techniques are expensive and time-consuming because of long sample preparation steps prior to analysis, and the functional analysis of new compounds synthesized cannot be examined *in vivo*. In order to overcome these problems, for the first time, a rapid, sensitive and inexpensive real-time biosensor system *in vivo* is introduced using intracellular reporter. In real-time biosensor system, because of the availability of a rapid and sensitive activity assay using biolominometer *in vivo* without cell lysis, luciferase has been considered as proper candidate.

Methods and Results: Co-transformation of the cells was carried out with two expression vectors containing Hsp70 and firefly luciferase, and the assessment of new drug compounds on Hsp70 activity under the stress conditions was evaluated. Our result showed that Hsp70 plays a crucial role in suppressing inactivation of luciferase *in vivo* during heat-treatment. On the other hand, the activity of heat-inactivated luciferase was approximately regained in cells expressing Hsp70 after incubation at room temperature for 60 min. According to the results, the reactivation of thermally inactivated luciferase was inhibited in the cells by treating with Ver-155008 and PFT-μ compounds (as apoptosis-inducing compounds) with IC_{50} of 124 and 384 μM, respectively. Also, the sensitivity of this method for detecting Ver-155008 and PFT-μ compounds was as low as 8 and 23 μM, respectively, and it showed no response to doxorubicin anti-cancer drug which binds to DNA, and used as control.

Conclusions: Real-time biosensor system using luciferase can be a class of *in vivo* biochemical tests used for simple, rapid and sensitive detection of apoptosis-inducing compounds based on Hsp70 inhibition to facilitate clinical and therapeutic studies in the years to come.

Key words: Drug Compounds, In Vivo, Hsp70, Luciferase, Co-Transformation