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Production design and generation of a whole-cell optic biosensor "Huh7-1X-ARE-luc"

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The effect of different toxins and various environmental stressors on cells can lead to the accumulation of free radicals in human body. These free radicals give rise to damages to cellular biopolymers through oxidative stress. Cell based biosensors refer to cells which have been genetically engineered to produce a measurable signal in response to specific chemical or physical agents in the environment. In this study, pGL4.26 plasmid, a vector containing the luc gene downstream of a minimal promoter was used as a reporter gene of the Huh7-1X-ARE-luc biosensor. Then the ARE fragment (antioxidant response element), which is expressed in response to oxidative stress was cloned upstream of luc gene as a response element of the biosensor. The ARE/Keap1-Nrf2 pathway is the major regulator of cytoprotective responses to oxidative stress. In order to investigate the sensitivity of the cell line, cells were incubated with increasing concentrations of a wide range of chemical food toxins inducing oxidative stress (i.e. lead). These toxins, enhance luciferase activity in a dose dependent manner, with sensitivity approximately about 30 μM lead concentrations as an oxidant agent. Real time PCR was performed on cells treated with 30 μM lead due to analyze NQO1 and NRF2 gene expressions. Based on our data, NQO1 and NRF2 expressions were upregulated in response to lead exposure, which confirmed the increment in our previous luciferase analysis. Finally, the specificity of the cell line was also accomplished by using non-oxidant specimens and the biosensor was able to differentiate toxic substances from non-toxic materials.

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