Apoptosome assay by Split-luciferase constructs

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Abstract

Introduction: Apoptosis is a process of programmed cell death that plays several critical roles in normal biological events happening in multi cellular organisms. Tissue homeostasis, defense against pathogens and involvement in development by controlling the number of cells are some of these critical roles. The two best-described activation mechanisms are the intrinsic (also called the mitochondrial pathway) and the extrinsic pathways. The formation of a supramolecular complex called apoptosome in mammals is tightly linked to ignition of the intrinsic pathway. This complex mainly consists of Apaf-1 molecules (apoptotic factor protease activating 1). The assembled Apaf-1 in apoptosome leads to the formation of functional caspase-9 that it further triggers the caspase cascade, a fundamental cascade that subsequently causes cell death. So detecting the formation of the apoptosome complex will help to screen the drugs and substances inducing intrinsic pathways also it helps in cell death related researches.

Methods and Results: we utilized previously developed split luciferase biosensor to investigate apoptosome activity of cells that were treated with Tunicamycin. Tunicamycin is an inhibitor of glycosylation that disturbs protein folding machinery in eukaryotic cells. Tunicamycin causes accumulation of unfolded proteins in cell endoplasmic reticulum (ER) and induces ER stress. ER stress is an essential mechanism for cellular homeostasis which has a role in cell death via reprogramming of protein processing, regulation of autophagy and apoptosis. Therefore, it can trigger apoptosis by induction of protein release such as cytochrome c that stimulates apoptosome formation. The biosensor consists of two separate constructs, N-terminal luciferase-Apaf-1 and C-terminal luciferase-Apaf-1. These constructs are cotransfected into mouse embryonic fibroblasts cells by polyethyleneimine (PEI). When apoptosome complex forms the assembling of Apaf-1 proteins brings the Nlucs and Clubs in spatial proximity that enables the enzyme to catalyst its substrate luciferin and bioluminescence. Split luciferase activity measured in several times after induction by Tunicamycin

Conclusions: apoptosome activity has fluctuation mode and we can control this complex activity by pharmacokinetic features of related drugs.

Key words: Apoptosis; Apoptosome; Apaf-1; Caspase; Split Luciferase; Doxorubicin