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MEETING ABSTRACT

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Real-time monitoring of apoptosis in live cells with FRET biosensor

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Background: Apoptosis or programmed cell death is a cellular defense mechanism, and its dysfunctions have been associated with several major diseases—for example, cancer. Apoptosis can occur via two different pathways, an internal pathway caused by cell stress or the external pathway triggered by ligand–receptor interaction. Both pathways activate a caspase cascade that leads to morphological changes and cell death. In the caspase family of proteases, there are seven caspases that are involved in apoptosis and can be classified as initiators (caspase-2, -8, -9, -10) or executioners (caspase-3, -6, -7) [1].

Methods: We have developed a method which uses FRET biosensor (Casper3-GR) and BacMam system for high-yield expression of sensor in both cancer and non-cancer cell lines [2]. When apoptosis is induced, time-dependent cleavage of the bond between two fluorescent proteins (TagGFP and TagRFP) is performed by caspase-3, leading to decrease in FRET. For measuring we use an automated microscope performing fluorescent imaging in two channels.

Results: Here, we show exhaustive validation of the method using HeLa cells and known apoptosis inducers like chemotherapy agents (bortezomib, MMAE) and compounds with a wide profile of potential targets (staurosporine). As a reference method, we used a viability assay utilizing resazurin dye which is reduced in live cells to fluorescent compound. By measuring data over 24 hours after adding the compound, we have found that different toxins have dissimilar FRET profiles in time, which reflects their efficiency and ability to modulate various cellular pathways.

Also, we could perform screening of novel as well as commercially available inhibitors of caspase-3—the compounds that can be viewed as protective agents against cell death. By inducing cell death with different toxins, we found that depending on the mechanism of binding of the caspase inhibitor (reversible *vs.* irreversible), the profile of FRET change is different.

Conclusions: In conclusion, we have developed a versatile method that combines advantages of FRET-based caspase-3 sensor and BacMam system. This method is easy to use, gives time-resolved information on the state of apoptosis in live cells, and can be combined with the 'classical' viability assays.

Keywords: apoptosis – FRET biosensor – caspase-3 – toxins – viability assay

References

1. Elmore S: **Apoptosis: a review of programmed cell death.** *Toxicol Pathol,* 2007; 35(4):495–516. doi:10.1080/01926230701320337

2. Mazina O, Allikalt A, Heinloo A, Reinart-Okugbeni R, Kopanchuk S, Rinken A: **cAMP assay for GPCR ligand characterization: application of BacMam expression system.** *Methods Mol Biol,* 2015; 1272:65–77. doi:10.1007/978-1-4939-2336-6_5

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