A Novel Cell-Based Drug Discovery Assay for Screening Modulators of Protein Aggregation and Toxicity

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ABSTRACT

Aggresomes are defined cellular organelles, often found in neurodegenerative diseases. Their role is not well understood, but it is believed that they serve as a storage site for toxic proteins and may be involved in the degradation of these proteins. Aggresomes are formed in response to some cellular stresses, such as hyperthermia, viral infection, or exposure to reactive oxygen species. They are formed by the accumulation of ubiquitin-conjugated proteins, which are then targeted for degradation by autophagy. A novel cell-based assay was developed to detect protein aggregation within cells using a novel red fluorescent molecular rotor dye. Aggresomes were generated using a variety of conditions known to induce protein aggregation and toxicity. The assay allowed assessment of the effects of protein aggregation directly in cells, without resorting to the use of non–heating conditions. The assay was validated with various potent, cell-permeable and selective proteasome inhibitors. The results suggest that the assay may be useful for the discovery of modulators of protein aggregation and toxicity.

BACKGROUND

In mammalian cells, aggregated proteins may lead to accumulation of cellular stress, such as hyperthermia, viral infection, or exposure to reactive oxygen species. These stresses can lead to the formation of aggregates, which can be detected using the described assay.

Molecular Rotor Dye: Molecular Rotor Mechanism

The described aggresome assay can be performed by fluorescence microscopy or by flow cytometry. ProteoStat™ dye provides a rapid, specific, and quantitative approach for detecting denatured protein cargo within autophagosomes, both in fixed and permeabilized cells. The described assay allows assessment of the effects of protein aggregation directly in cells, without resorting to the use of non–heating conditions.

ProteoStat™ Dye Detects Protein Aggregate Accumulation within Aggresomes

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Cell Aggresome Assay Protocols

The described assay allows assessment of the effects of protein aggregation directly in cells, without resorting to the use of non–heating conditions. The assay was validated with various potent, cell-permeable and selective proteasome inhibitors. The results suggest that the assay may be useful for the discovery of modulators of protein aggregation and toxicity.

CONCLUSIONS

ProteoStat™ dye can provide a rapid, specific, and quantitative approach for detecting denatured protein cargo within autophagosomes in fixed and permeabilized cells. The described assay allows assessment of the effects of protein aggregation directly in cells, without resorting to the use of non–heating conditions. The assay was validated with various potent, cell-permeable and selective proteasome inhibitors. The results suggest that the assay may be useful for the discovery of modulators of protein aggregation and toxicity.