ABSTRACT

Aggresomes are inclusion bodies that form when the ubiquitin-proteasome machinery is overwhelmed with aggregated proteins. Aggresomes form in response to cellular stress, such as hyperthermia, viral infection or exposure to reactive oxygen species. Aggresomes may provide a cytoprotective function by sequestering the toxic, aggregated proteins and may also facilitate their elimination from cells by autophagy. Certain cellular inclusion bodies associated with human disease are thought to arise from an aggresomal response, including Lewy bodies in Parkinson’s disease and muscle inclusions in alcoholic liver disease. A heterogeneous fluorescence-based assay was devised to detect aggresomes within cells. The assay was also performed using a panel of proteasome inhibitors. The assay demonstrated the ability to detect aggresomes in a cellular context, employing a novel red fluorescent molecular motor dye. With minor modification, the assay is also suitable for analysis of tissue sections. This assay has been validated using a range of conditions known to modulate proteasome pathways and has been optimized for cell-localization studies with fluorescently labeled antibodies, highlighting interesting differences between the cytoplasmic and nuclear compartments. Furthermore, the novel assay has been applied to screen for drugs that promote autophagy or prevent aggresome formation, such as pLL and L33. This assay is compatible with flow cytometry, allowing, for the first time, easy quantification of destabilized protein cargo. Furthermore, antibody to a tau peptide 1-42 was shown to reduce aggregation formation in the SK-N-SH human neuroblastoma tumors cell line. SMER28, a small molecule modulator of autophagy, is an mTOR-independent modulator, increasing detection of antibody peptide within the cells. The described assay circumvents assessment of the effects of protein-aggregation in directly cells, without resorting to the use of non-physiological protein mutants or genetically engineered cell lines.

Detecting Aggresomes by Gel Electrophoresis/ Western Bloting

- Developing agarose gel does not create proteoglycan aggregates.
- Migrates molecular weight standards of Ab1, 150M daltons in the presence of 10% SDS and 0.1M Tris-Cl, pH 6.8 and 10% glycerol should be loaded on the gel.
- The subproteome for detection between the histidine/arginine residues.
- Type II & III muscle Inclusions (Harley et al. 1977). 30-50 DD.

Analyzing Aggresomes and Aggresome-like Inclusions Bodies in Cells by Fluorescence-Based Assay

- Cells are fixed, allowing for co-localization studies with fluorescently labeled antibodies.
- The nucleus (N) and mitochondria (M) are also visible.
- The aggresome has three 'lobes', one of which surrounds a centriole (c).
- Block aggresome formation does not prevent aggresome formation.
- Different biochemical assays generate different compound leads.
- Old paradigm: Screen biochemically, then test in animals.
- Testing Other Modulators of Autophagy and Degradation by Flow Cytometry

Conclusions

- Protist® Aggresomal assay provides a rapid, specific and quantitative approach for detecting destabilized protein cargo in fixed and permeabilized cells.
- The assay can be performed by fluorescence microscopy or by flow cytometry.
- The assay has been validated with small molecule modulators known to influence autophagy and proteasome pathways.
- The assay is suitable for co-localization studies with fluorescently labeled antibodies or highlighting interactions between aggregated protein cargo and proteins implicated in aggregation formation, such as pLL and L33.
- This assay enables screening of aggregation inhibitors, relevant to neurodegenerative disease, in an authentic cellular context.

Figure 1. Aggregated protein cargo is detected by ProteoStat® dye as observed by fluorescence microscopy. HeLa cells were mock-induced with 0.2% DMSO (a) or induced with 5 μM MG-132 (b) for 30 minutes at 37°C. After treatment, cells were fixed and incubated with ProteoStat® dye, then analyzed by flow cytometry.

Figure 2. M. renna et al. Nat. Chem. Biol. 1. S. Sarkar peptide with 5 μM SMER28 (d) 25 μM amyloid beta peptide with 50 μM SMER28.

Figure 3. 0.5 μM Epoxomicin (c) 4 μM Lactacystin and (d) 0.5 μM Velcade® with overnight incubation.

Figure 4. MG-132 Selective proteasome inhibitor Activates autophagy N/A 1~4 HeLa, HepG2, MG-132 activates autophagy 4~10 18 HeLa, Jurkat

Figure 5. Different biochemical assay generate different compound leads.

Figure 6. A. 5 µM MG-132 activates autophagy. Results are presented as histograms for the % treated cell signal, compared to signal from untreated cells, normalized to signal increase over basal.