A Fluorescence-Based Sensor Assay that Monitors General Protein Aggregation in Human Cells

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The accumulation of protein aggregates is a common feature of protein conformational disorders and is generally correlated with the onset of diseases, such as amyotrophic lateral sclerosis (ALS), Alzheimer's (AD), Parkinson's (PD), Huntington's (HD) disease and other age-related diseases (ARD).

Different in vitro and in vivo protein misfolding/aggregation monitoring strategies have been developed in recent years. However, most of the available protein aggregation sensors are limited to monitor a single class of proteins. With this in mind, we developed a protein aggregation sensor that can be used to monitor general protein aggregation in human cells and we have established a stable cell line to test it in multiple physiological conditions. For this, we took advantage of the observation that the small heat shock protein 27 (HSP27) is efficiently recruited by misfolded proteins (Figure 1-A). HSP27 binds to misfolded proteins forming a chaperone-substrate complex that permits refolding by larger ATP-dependent chaperones, namely HSP70 and HSP90 or directing proteins for degradation. Our data show that this sensor can detect a wide range of protein aggregates in cells and is a valuable tool to identify different protein misfolding/aggregation promoting conditions (Figure 1-B). One of the main features of this sensor is that it can be used in in vivo testing, allowing monitoring protein aggregation in a time dependent manner. We are currently using this protein aggregation sensor to perform high content screenings to identify genes that are essential for proteostasis and can therefore represent promising therapeutic targets for diseases where protein homeostasis is compromised. This sensor is also being used in drug screenings to identify drug candidates that modulate protein aggregation, representing a powerful tool in preclinical stages of the drug discovery pipeline.

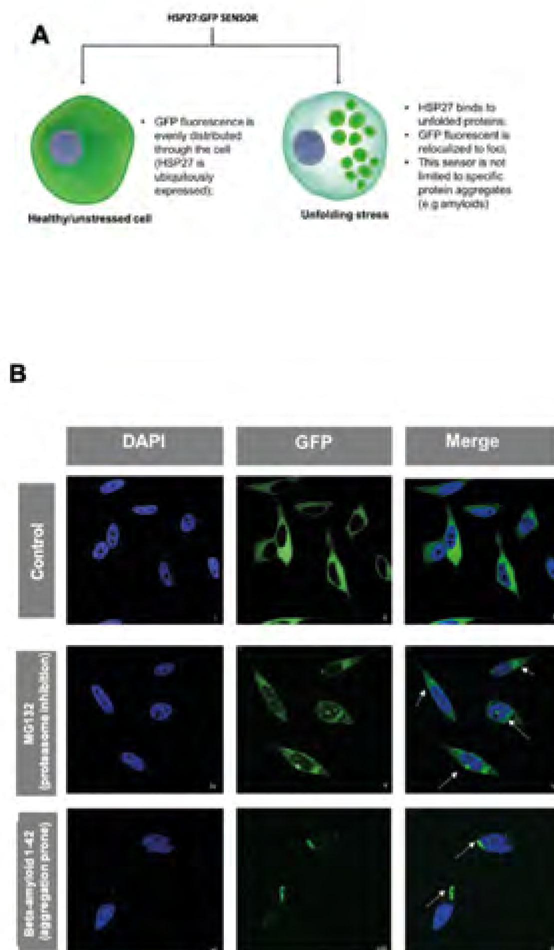

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FIGURE 1

HSP27:GFP detects general protein aggregation. A) HSP27:GFP sensor description. The HSP27:GFP protein aggregation sensor relies on the ability of HSP27 chaperone to be recruited to misfolded proteins. In healthy/unstressed cells, GFP is evenly distributed throughout the cell, as HSP27 is ubiquitously expressed. After exposure of cells to unfolded stresses, GFP fluorescence is relocalized to foci corresponding to misfolded proteins locations, as HSP27 is recruited to misfolded proteins to either stimulate their refolding, or target them to degradation. B) Confocal microscopy of HeLa cells expressing the HSP27:GFP sensor. HeLa HSP27:GFP cells were fixed, the nuclei stained with DAPI and observed under a Zeiss Confocal microscope in control conditions (i, ii, iii), after proteasome inhibition (iv, v, vi) and after incubation with an aggregation prone amyloid peptide (Aβ-1-42) (vii, viii, ix). In control conditions (i, ii, iii), the fluorescence is evenly distributed throughout the cells, as expected. After inhibition of the proteasome there is accumulation of misfolded proteins in the cytoplasm, as they are not actively being degraded. This accumulation is detected by the HSP27:GFP sensor (arrows) (iv, v, vi). The aggregation prone amyloid peptide (Aβ-1-42) is also detected by the protein aggregation sensor (arrows) (vii, viii, ix). Images were captured in separate channels using different lasers (Diode for DAPI and Argon for GFP detection) and merged using Image J.