Fluorescence-based assay development to screen drug against Amyotrophic Lateral Sclerosis disease

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Abstract

Amyotrophic lateral sclerosis (ALS) is one of the most common degenerative disease of the motor neuron system.The TDP-43 pathology seems to be a dominant type of pathology across sporadic ALS types. Innoprot has developed a novel fluorescence cell-based assay for High Content Screening that allows the quantification of pathological TDP43 globs into the nucleus and cytosol . In this work we used this model to screen a library of 57 compounds using Arimoclomol as a positive control. After the screening campaign, 10 compounds were chosen for further testing, based on the strength of the initial response and lack of cytotoxicity. Our results indicated that the pharmacological inhibition or modulation of TDP43 globs formation implicated in ALS remains a valid strategy for drug screening.

Results

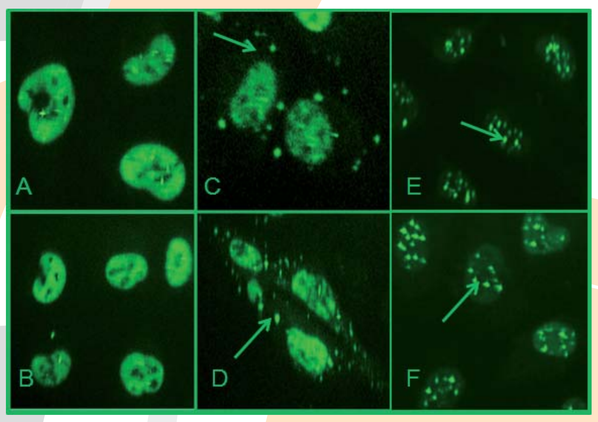

Fig.1.Cellular fluorescence redistribution after sodium arsenite treatment. Representative images of the negative controls show a nuclear distribution of the fluorescence (A,B). However, after sodium arsenite treatment the phenotype turns into a cytosolic vesicular pattern corresponding to stress granules (C,D) and into an intensive nuclear globs pattern (E,F).

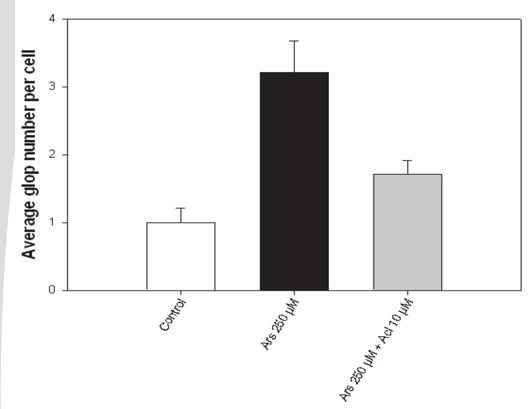

Fig.3. Protective effect of Aromoclimol againts oxidative stress. After TDP43-tGFP expression induction, the cells were incubated with Arimoclomol at 10 uM during 24 hours. Then, the cells were treated with 250 uM sodium arsenite during 90 min. The TDP43-tGFP nuclear globs were quantified using the BD Pathway HCS Reader and Attovision Compartimentalization Software. Error bars represent the standard deviation among 3 replicate wells.

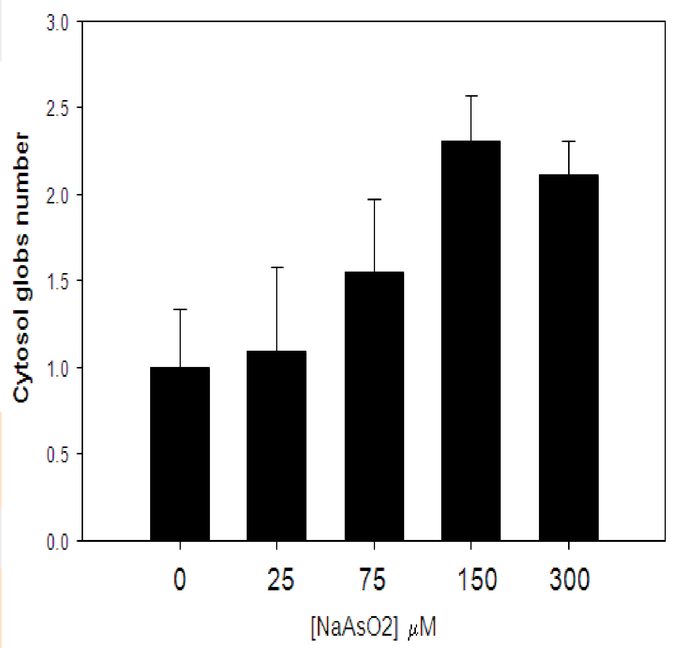

Fig.2. Cytosolic stress granules appearance after sodium arsenite treatment. Cellular model was treated with 5 mM of IPTG during 72 h to induce the TDP43-tGFP expression. After that, the cells were treated with a range of sodium arsenite concentrations from 25 to 300 uM during 90 min. The cytosolic TDP43 containig stress granules was quantified using the BD Pathway HCS Reader and Attovision Compartimentalization Software. Error bars represent the standard deviation among 3 replicate wells.

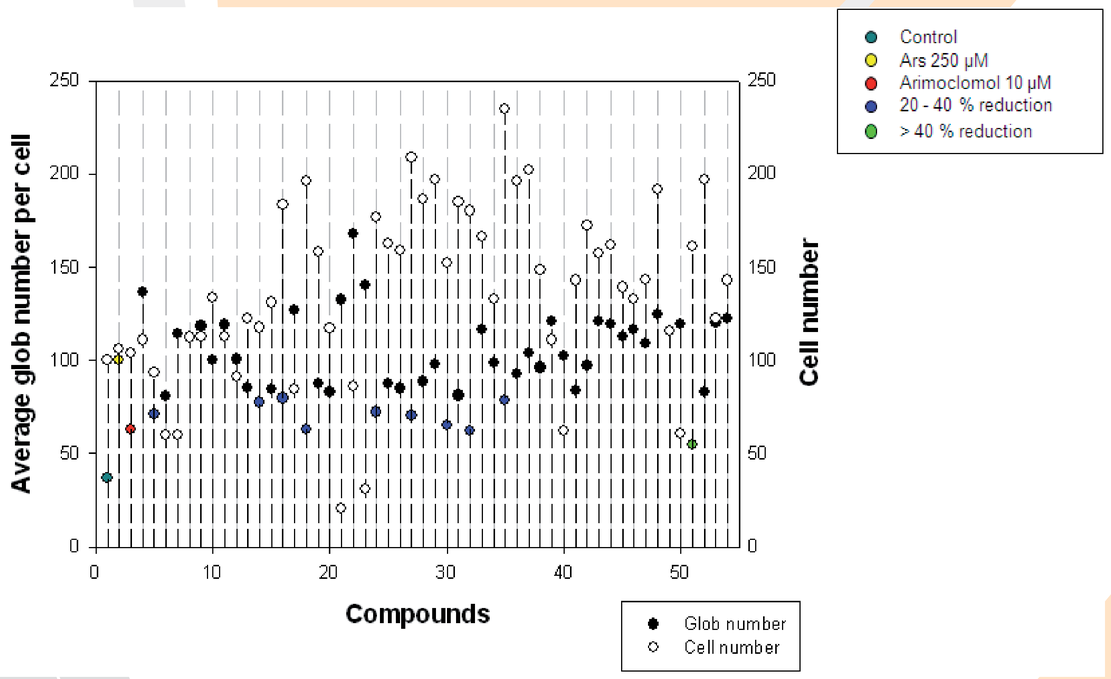

Fig.5. Compounds toxicity. The number of globs (black) is represented related to the cell number (white). The results show the compounds that the ten positive compounds (Fig.4) did not affect the cell viability, so they were selected for further studies.

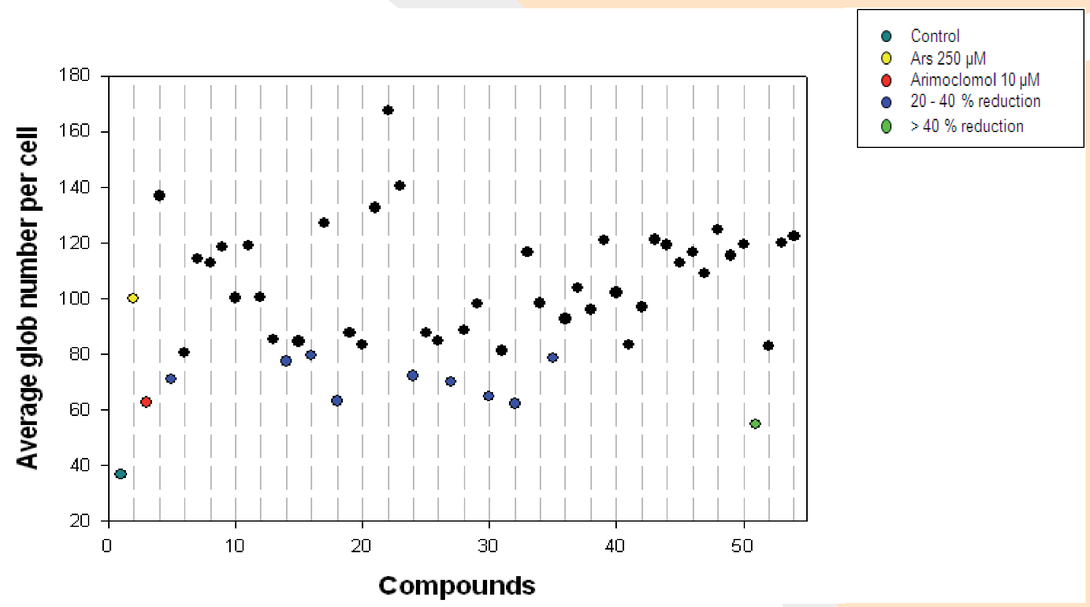

Fig.4. Screening of 57 compounds. Before oxidative stress induction by sodium arsenite, the cells were incubated with the compounds at 10 mM during 24 hours. Then the nuclear glob number was quantified using Attovision software . The control of TDP43-tGFP expressing cells is represented in green. The positive control (Arimoclomol) is represented in red and the negative control (Sodium arsenite) is represented in yellow. The blue spots represent compounds that show a nuclear globs numbers reduction around 20-40%. The light -green spots represent compounds that show a nuclear globs numbers reduction >40%.

Methods

Cultured cells: The U2OS cell line has been used for constitutive and inducible cell line generation. The cell line was cultured into 96 wells Imaging Plates BD at 4000cell/well in 200 ul of DMEM F12 10% FBS and incubated at 37 ºC and 5 % CO2.

Image acquisition: Cell line stably expressing human tagged TDP43 was treated with Sodium arsenite during 90 min. After that, the TDP43 inclusions presence in the cytosol and in the nucleus was quantified by fluorescence using image analysis algorithms.

Conclusions

1.-The stably transfected TDP43 cell line can be used in drug discovery for pathological globs formation inhibitors.
2.-This model permits to evaluate the TDP-43 protein distribution in living cells studying the protein localization pattern in the space and time.
3.-This model provides a strategy to evaluate drugs that not have cell permeability.
4.- This cellular model have been adapted to HCS analyses based on image algorithms to test cytosolic and nuclear globs generation process.