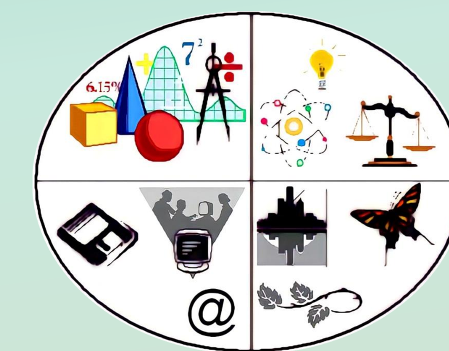
Identification of stress granule modifying compounds in cancer cell lines by high-content screening (HCS) microscopy



István Révész

Bessenyei György Secondary Grammer School, Kisvárda, Hungary

Introduction

Stress granules (SG) are membrane-free cytoplasmic organelles arising in cells exposed to stress. They are condensates of proteins and RNA. SGs regulate translation, mRNA storage, the antiviral response and signaling pathways, through which SGs promote stress adaptation. Cancer cells take advantage of SG formation to evade programmed cell death, to metastasize and to acquire resistance to chemotherapy. Although SGs are potential drug targets in cancer, there are no specific SG inhibitors. Their development requires the identification of starting compounds. To fulfill this objective, we are planning to set up a high-throughput chemical compound screen (HTS). The identified molecules can, potentially, have future applications in cancer therapy or provide molecular targets for the design of such agents (Asadi et al., 2021).

Research methodology

Labeling of stress granules: We acquired the cDNA of the RNA-binding protein, G3BP1, an essential component of SGs, labeled with the enhanced green fluorescent protein (EGFP) by gene fusion (Figure 1a).

Cell model generation: U2OS and HeLa cell lines were transfected with the plasmid by lipofection. Cells having integrated the fusion gene construct stability were selected using geneticin (Figure 1b).

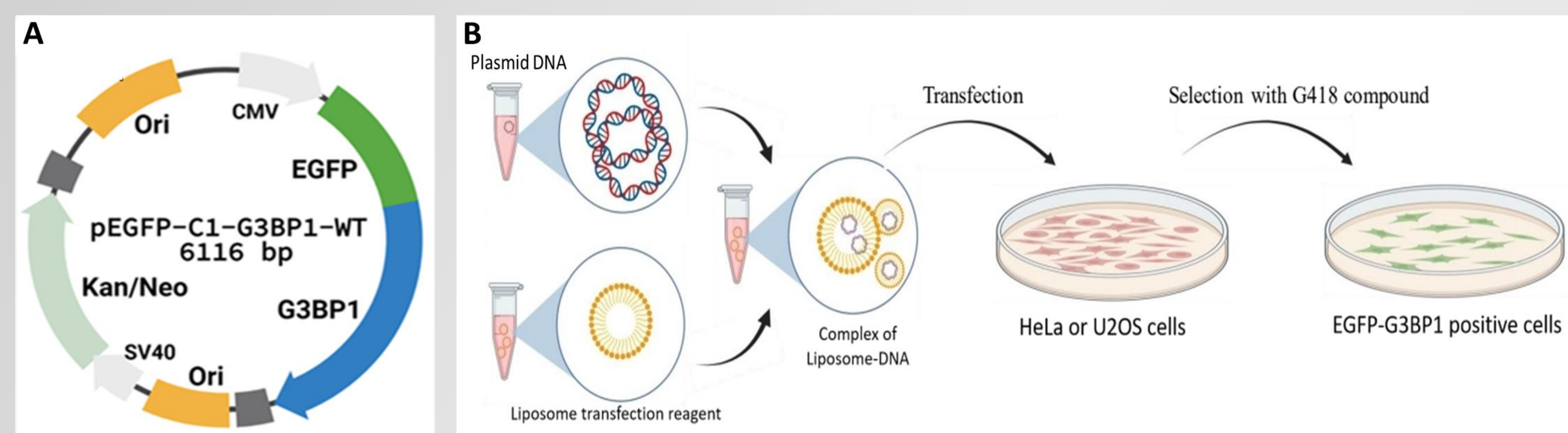
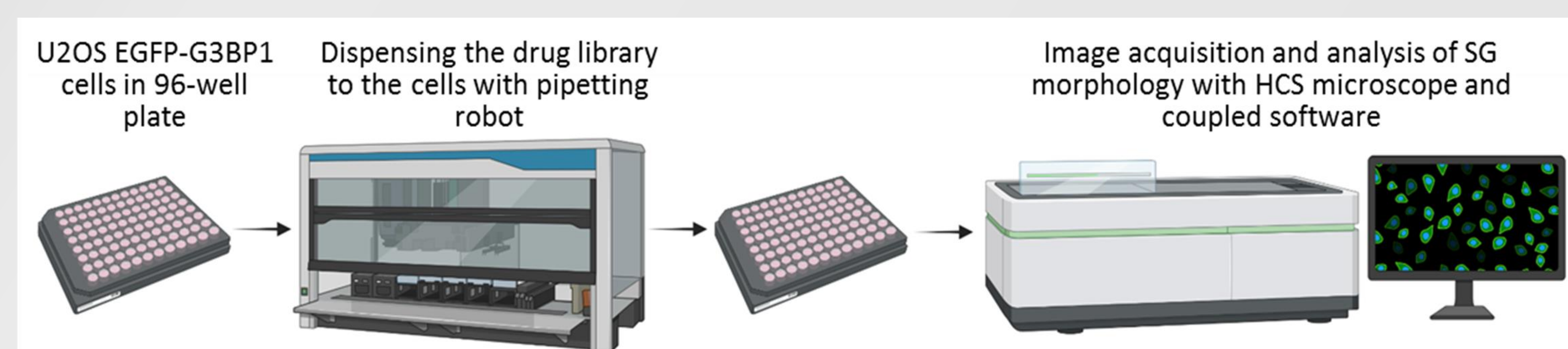


Figure 1 – Map of the EGFP-G3BP1 plasmid and outline of the lipofection and selection

Drug screen: The cells were plated into glass-bottom 96-well cell culture plates. Prior to the screening, they were labeled with CellTracker Blue reagent to aid in finding cell boundaries. Compounds from the Screen-Well® FDA Approved Drug Library-V2 were dispensed into the wells with the help of a pipetting robot at 10 µmol/dm³. After 1 hour of pre-incubation, cellular stress was triggered with sodium arsenite for another hour. The cells were fixed with paraformaldehyde and images were acquired with an Opera Phenix HCS microscope (Figures 2).



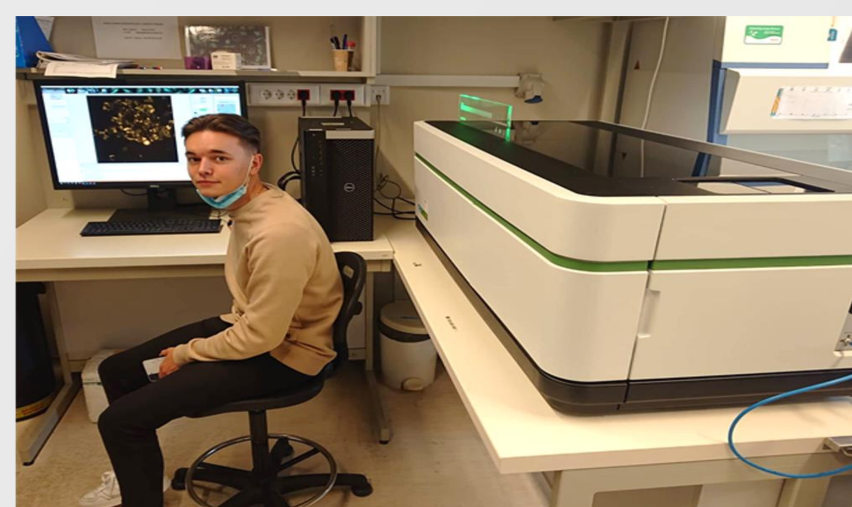


Figure 2 – Flow diagram of the drug screening and the author with the instruments in the Medical Chemistry Department of University of Debrecen

Image analysis: Perkin Elmer's Harmony software was used to analyze the images. Cell boundaries were identified based on the CellTracker Blue staining and SGs as high-intensity EGFP positive speckles.

Results and Discussion

The expression of the EGFP-G3BP1 fusion protein was verified by fluorescent microscopy. The fusion protein responded to stress triggered with 0.5 µmol/dm³ sodium arsenite and accumulated in SGs (Figure 3).

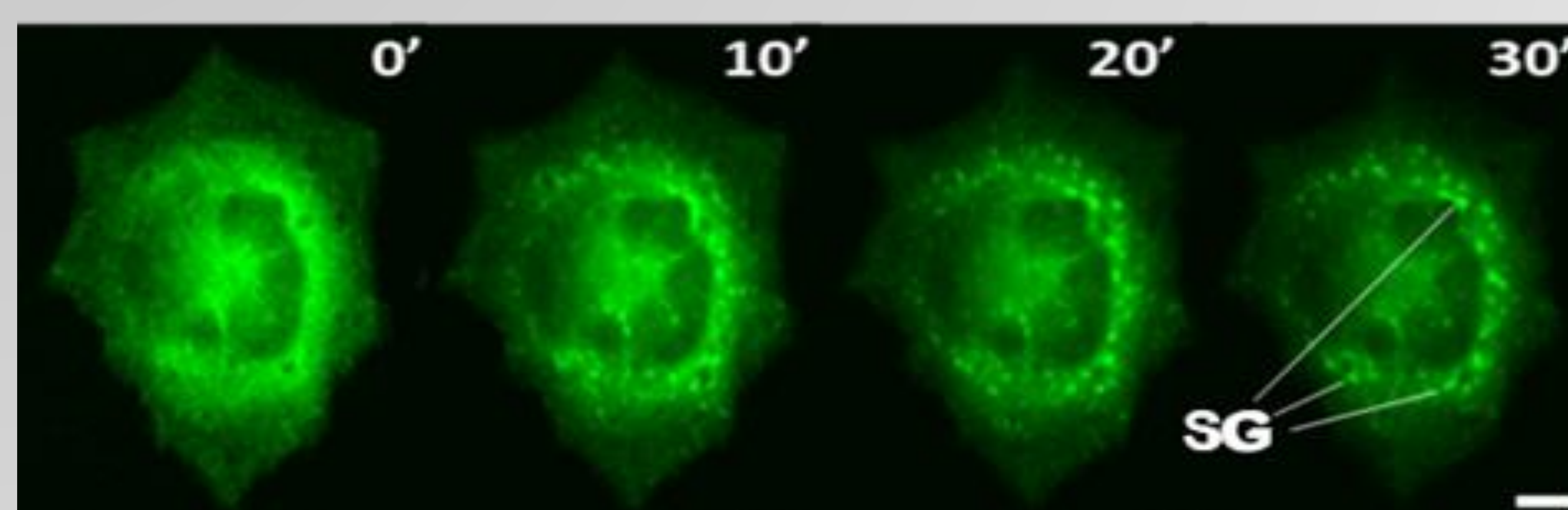


Figure 3 – Rapid relocalization of EGFP-G3BP1 into SGs upon stress (0-30 min). Scale bar: 10 µm

In this research work, a processing pipeline was created to segment the images into areas corresponding to cells and to identify the EGFP-labelled SGs (Figure 4).

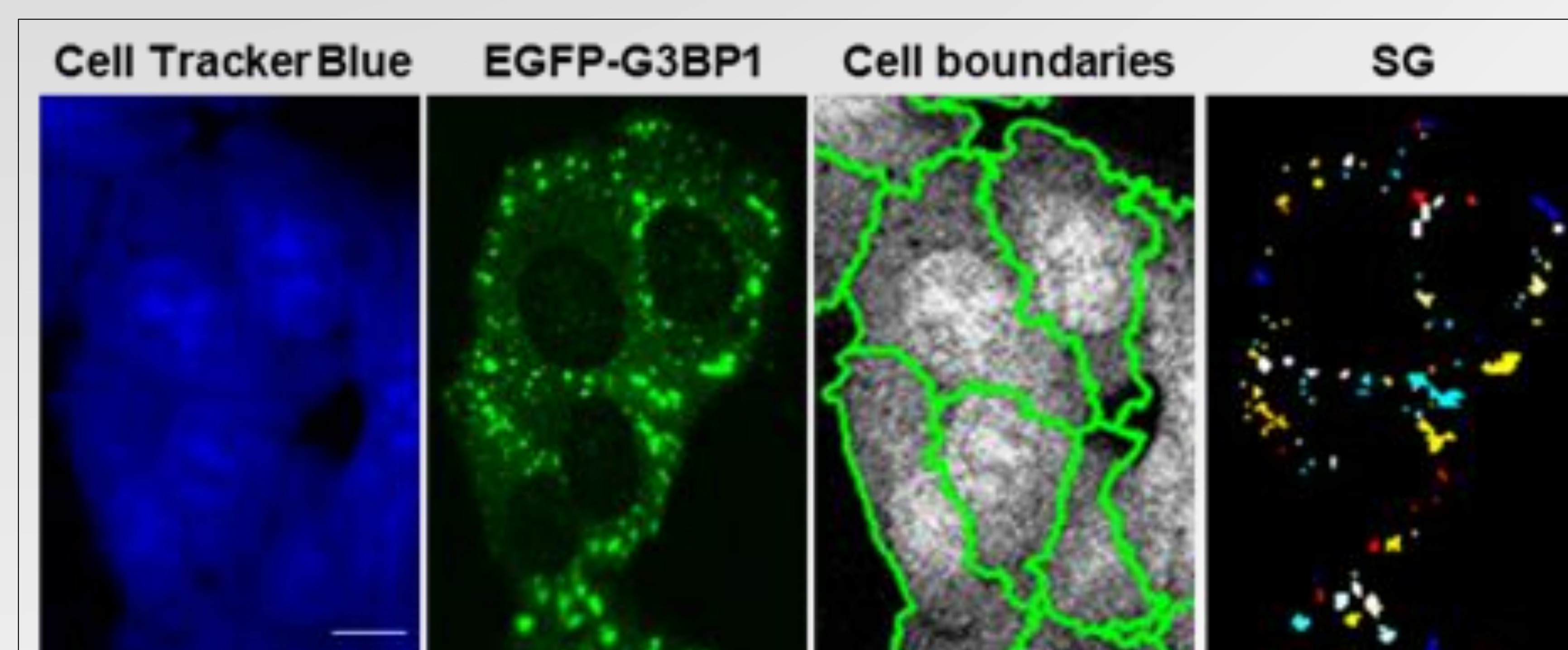


Figure 4 – Identification of the cells and the SGs in the microscopy images by the software. Scale bar: 10 µm

Conclusion

A stably transfected cancer cell model was generated to study SGs by microscopy and an image analysis pipeline was set up to identify SGs in images. The model system can be used to screen for compounds preventing SG formation or altering SG morphology.

Future work

Images of cells treated with ~1/10 of the compounds in the library have already been collected. The image analysis is underway and will be completed soon. Afterward, the rest of the compounds will be tested. Finally, selected compounds with an inhibitory effect on SG formation will be studied individually to determine their effect on cell viability, survival and stress tolerance.

References

Asadi, M.R., Rahmanpour, D., Moslehian, M.S., Sabaie, H., Hassani, M., Ghafouri-Fard, S., Taheri, M., & Rezazadeh, M. (2021). Front Cell Dev Biol. 9:745394. doi: 10.3389/fcell.2021.745394.