Project Example

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- **Project Title: Spatial proteomics of stress granules**
- **Project Description**

I. Background

Stress granules (SGs) are dynamic, non-membrane-bound assemblies of proteins under unfavorable environmental stresses such as toxins, hypoxia, oxidative stress, and temperature changes. Eukaryotic cells form SGs in the cytosol to protect against damage and promote cell survival. Serval diseases including cardiovascular disease [1], neurodegenerative disorders [2,3], viral infection [4], aging [5] and cancer [6] have been identified to be associated with SGs. Emerging evidence has shown that the assembly and disassembly of SGs determine protein storage, translation remodeling, and degradation of untranslated mRNA, which can lead to cell survival and increase resistance to stress and drugs [7].

It has been technically challenging to isolate stress granules from the cell for proteomic study due to their small-size and membrane-less nature. Recent SG proteomic studies have been achieved by genetic fusion construct followed by proximity labeling [8]. The requirement of genetic fusion construct is limited and tedious with non-specific expressions, thus the

composition and dynamic assembly of SG remains poorly understood. Overall, targeting SGs is a potential therapeutic strategy to discover novel stress granule proteins, understand the dynamic and composition of SG formation, and a promising treatment for diseases and the promotion of health.

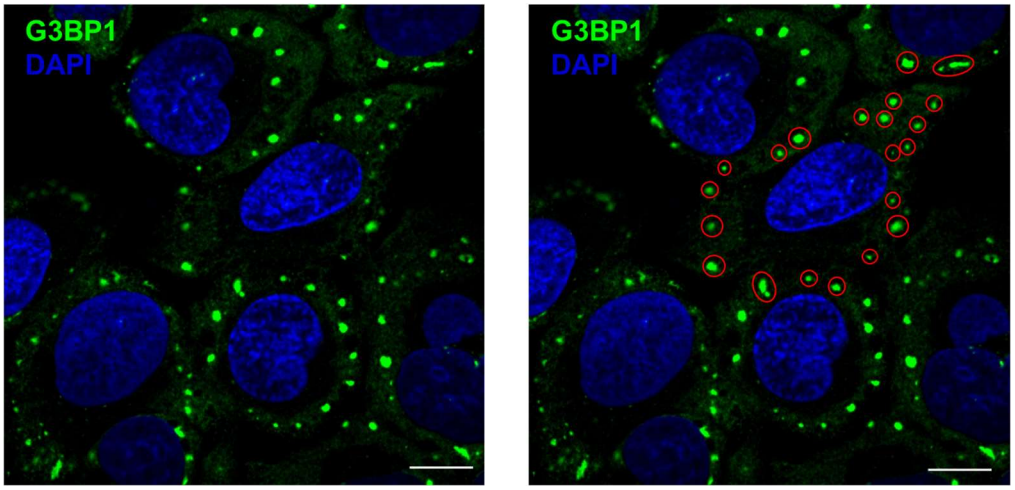

Figure 1. Stress granules (G3BP1: green) are induced by arsenite for 1 hour in U-2OS cells (left). Circles in red are the regions of interest for SG proteome (right). Scale bar: 10 μM.

II. Specific Aim(s)

SGs will be first induced in U-2OS cells by arsenite then immunostained with a stress granule marker, G3BP1. Immunofluorescence images of G3BP1 will be applied to generate a computer vision (CV) based algorithm to differentiate G3BP1 in the cytosol as our targets. Then, the recognized targets will be subject to Microscoop to process photo-induced biotinlylation. Sequential process of photo-induced labeling will be processed automatically to achieve target protein-specific biotinylation until sufficient cells are labeled for streptavidin-based pulldown and subject to LC-MS/MS analysis.

III. Sample information

Sample type: U-2OS Cell
Treatment: Arsenite

Preparation: methanol fixation
Imaging channel(s): anti-G3BP1 antibody, Alexa 647

IV. Significance

Stress granules are membrane-less protein aggregation in the cytosol of a stressed cell for its survival and storage, therefore, a better understanding of SG formation and composition may shed light on the development of novel therapeutic targets. With the benefit of the Microscoop, it would be capable to label a size less than 1 µm stress granules for hypothesis-free biomarker discovery. It is therefore possible that clearance the abnormal SG protein aggregation or targeting novel SG proteins may be a promising therapeutic strategy in diseases and cancer.

V. References

[1] Herman A, et al. (2019). Regulation of stress granule formation by inflammation, vascular injury, and atherosclerosis. Arterioscler Thromb Vasc Biol 39: 2014‐2027.
[2] Patel A, et al. (2015). A Liquid‐to‐Solid phase transition of the ALS Protein FUS accelerated by disease mutation. Cell 162, 1066‐1077.
[3] Wolozin B, et al. (2019). Stress granules and neurodegeneration. Nat. Rev. Neurosci 20, 649–666.
[4] McCormick C, et al. (2017). Translation inhibition and stress granules in the antiviral immune response, Nat. Rev. Immunol. 17 (2017) 647–660.
[5] Cao X, et al. (2020). The involvement of stress granules in aging and agingassociated diseases. Aging Cell 19, e13136.
[6] Gao X, et al. (2019) Stress granule: a promising target for cancer treatment, Br. J. Pharmacol 176, 4421–4433.
[7] Wang F, et al. (2020). Targeting stress granules: A novel therapeutic strategy for human diseases. Pharmacological Research. 161, 105143.
[8] Marmor-Kollet H, et al. (2020). Spatiotemporal Proteomic Analysis of Stress Granule Disassembly Using APEX Reveals Regulation by SUMOylation and Links to ALS Pathogenesis. Mol Cell 80(5), 876-891.