PhD DEFENCE – Wednesday, June 22nd, 2016

Student: Gabriel Fung

Title: **Interplay Between Stress Granules, Cellular Stress Response, and Coxsackievirus B3 Infection**

Time and Location: 12:30 PM PDT, Gourlay Conference Room (CHLI), St. Paul's Hospital, Burrard Building, 1081 Burrard St., Vancouver, BC

Supervisor: Honglin Luo

ABSTRACT

Viral infection affects a multitude of cellular processes to facilitate successful replication. Such responses include the formation of stress granules (SGs) and the activation of autophagy. SGs are stalled translational complexes and function to restore cellular homeostasis after stress. Autophagy is a cellular process that recycles misfolded proteins and damaged organelles and plays an important role in various stress responses. We previously demonstrated that infection with Coxsackievirus B3 (CVB3), a common human pathogen for viral myocarditis, disrupts the autophagic process to support effective viral replication. However, the interplay between CVB3 and SGs, and the ability of SGs to regulate autophagy have not been investigated. Here we showed that SGs are formed early and actively disassembled late during CVB3 infection due to viral protease 3Cpro-mediated cleavage of Ras-GAP SH3 domain binding protein 1 (G3BP1), a key nucleating protein of SGs. Overexpression of G3BP1 inhibits CVB3 replication, indicating an anti-viral function of SGs. We further demonstrated that the C-terminal product of G3BP1 has a toxic gain-of-function that further inhibits SG formation. We also examined the interaction between CVB3 and the transactive response DNA-binding protein-43 (TDP-43), an RNA binding protein that mislocates to SGs under cellular stress. We found that TDP-43 is translocated from the nucleus to SGs upon infection through the activity of viral protease 2Apro, followed by the cleavage by protease 3Cpro. The C-terminal product of TDP-43 is quickly degraded by the proteasome, whereas the N-terminal truncate acts as a dominant-negative mutant that inhibits the function of native TDP-43 in alternative RNA splicing. Knockdown of TDP-43 results in an increase in viral titres, suggesting a protective role for TDP-43 in CVB3 infection. Lastly, we explored the possible role of G3BP1-SGs in regulating autophagy. We showed that G3BP1 inhibits autophagic flux, likely by binding to cytoplasmic signal transducer and activator of transcription 3 (STAT3). Taken together, our results reveal that the host SGs and associated proteins, including G3BP1 and TDP-43, are utilized and modified during CVB3 infection to promote efficient viral replication and induce viral pathogenesis. Moreover, we propose a novel mechanism by which G3BP1 binds cytoplasmic STAT3 to inhibit autophagy.