PhD DEFENCE Wednesday, February 27th, 2019
Student: Charles Soong
Title: SIGNATURES OF GENOMIC INSTABILITY AND DNA REPAIR DEFECTS AS DETERMINANTS FOR TUMOUR CLONAL DYNAMICS AND SENSITIVITY TO G-QUADRUPLEX STABILIZERS
Time and location: 9:00 AM; Room 200, Graduate Student Centre, 6371 Crescent Road, UBC Point Grey Campus, Vancouver, BC
Supervisor: Dr. Sam Aparicio

ABSTRACT

Mutational processes, including DNA repair defects, have been extensively documented across multiple cancer types. Targeting such defects has yielded much promise in the field. Previous studies have shown great therapeutic potential in targeting G-quadruplex (G4) structures in HR (homologous recombination) and NHEJ (non-homologous end-joining)-deficient tumour cells with the G4 stabilizer, CX-5461. In this thesis, we hypothesized that additional genome maintenance pathways and genes may also be relevant in repairing lesions from G4 stabilization.

First, we performed an *in vitro* subgenome-wide CRISPR/Cas9 screen targeting 480 genome stability-associated genes, in HCT116 colorectal carcinoma cells treated with CX-5461, pyridostatin (PDX; a known G4 binder), and BMH-21 (a non-G4 binder). We discovered novel G4-associated genes and pathways including nucleoplasm, DNA secondary structure binding, and ubiquitin signaling. In addition, we identified DNA polymerase theta (POLQ), known to have roles in the microhomology-mediated end-joining (MMEJ) pathway which is commonly thought to act as backup repair to HR and NHEJ, as a top depleted gene. G4 stabilizers sensitized POLQ deficient cells in multiple cell lines. Next, we studied *in vivo* the competitive dynamics of cell populations harbouring different sgRNA-induced DNA repair defects in both cell line- and patient derived xenografts (PDX). Particularly in cell line xenografts, CX-5461 led to the specific lethality of HR (BRCA2) and MMEJ (POLQ) deficient subpopulations, underscoring the importance of both pathways for G4 stabilization *in vivo*. Finally, we assessed the mutations and changes in DNA repair patterns caused by G4 stabilization. From WGSS and targeted probe capture sequencing, we observed an increase in mutations in drug-treated cells. Furthermore, using a sequencing based assay to measure different DNA repair pathways, we discovered that in NHEJ-depleted cells, cells preferentially used POLQ-mediated MMEJ, rather than HR, to repair DNA lesions caused by G4 stabilization.

Altogether, this study discovered a spectrum of additional genome maintenance-associated vulnerabilities that could be targeted with G4 stabilizer drugs for cancer treatment. In addition, this study identified the novel role of POLQ in repairing DNA damage induced by G4 stabilizers both *in vitro* and *in vivo*.