Name of Student: Chi-Chao Liu

Research Supervisor: Chinten James Lim

Title of Presentation: Calreticulin interaction with the membrane-proximal KXGFFKR motif of α-integrin mediates chemoresistance in T-cell leukemia

Abstract

Acute lymphoblastic leukemia is the most prevalent malignancy in children. Current chemotherapy-based treatment regimes achieve 5 year survival rates of 80% for children and 50% for adults. However, the prognosis for relapsed chemoresistant leukemia remains dismal (20-50%).

Cell adhesion mediated drug resistance is a major contributor to relapse in hematological malignancies. In published work (Mol Cell Biol), we showed that Jurkat T-leukemia cell adhesion to α4β1 or α5β1-integrin substrates promotes chemoresistance. Expression of a truncated mutant α-integrin, bearing only KXGFFKR as the cytoplasmic motif, promoted chemoresistance in an integrin adhesion-independent manner. Using this adhesion-independent gain of chemoresistance cell model, we characterized several cell survival phenomenon that is constitutively activated in the truncated α-integrin mutant otherwise normally triggered by cell adhesion. This includes activation of Akt, increased influx of extracellular Ca^{2+} and increased efflux of cytotoxic agents. Most significantly, the multi-functional Ca^{2+}-binding protein calreticulin was constitutively associated with the mutant integrin via the KXGFFKR motif, while α4β1-mediated adhesion promoted the calreticulin-integrin interaction. These findings stressed the role of calreticulin in α-integrin KXGFFKR motif mediated chemoresistance in leukemia.

Recent work done by Nangalia *et at* and Klampfl *et al* (NEJM) identified calreticulin as the most frequently occurring somatic mutation in myeloproliferative neoplasms after JAK. The mutations cluster near the C-terminus and produce novel C-terminus missing the KDEL ER retention motif. Overexpression of the mutant calreticulin promoted JAK-STAT signaling and cytokine-independent proliferation. We postulate the mutant calreticulin may be enriched in the cytosol, where its interaction with integrins forms an oncogenic switch that drives survival signaling and cell proliferation.

To investigate the role of calreticulin, and in particular, the cytosol enriched-form of calreticulin in cell adhesion dependent and independent chemoresistance, we generated calreticulin$^{-/-}$ cells by CRISPR-Cas mediated gene silencing, and show that calreticulin is implicated in integrin activation, cell adhesion, and enhanced resistance to chemotherapy. We found that IL-7 stimulated phosphorylation of STAT3 is abrogated in calreticulin$^{-/-}$ Jurkat cells. Furthermore, knockdown of calreticulin expression in cells expressing the truncated mutant integrin led to reduced STAT3 phosphorylation even in the presence of cytokine stimuli. Taken together, **Calreticulin interaction with the membrane-proximal KXGFFKR motif of α-integrin mediates chemoresistance possibly through JAK-STAT signalling.**