Title: A potential role of alternative splicing control in DNA damage response.

Authors: Chang YC and Nguyen HD

Myelodysplastic syndrome (MDS), a heterogeneous group of clonal hematopoietic stem cell disorders, is a common adult myeloid malignancy that can transform into chemo-resistant secondary acute myeloid leukemia (sAML). Somatic heterozygous mutations in spliceosome (SF) genes (SF3B1, U2AF1, SRSF2, and ZRSR2) occur in over 50% of MDS patients and are mutually exclusive. Among these mutations, the hotspot mutations (S34 and Q157) in U2 small nuclear RNA auxiliary factor 1 (U2AF1), which causes mis-splicing, are associated with worse overall survival and an increased risk of transformation to sAML. Additionally, about 50-60% of MDS patients have chromosomal instability, indicative of deficits in genome maintenance pathways. Interestingly, U2AF1 phosphorylation at S59 increases upon etoposide treatment. Whether U2AF1 bridges the alternative splicing and DNA damage response (DDR) in cells remains elusive.

We aim to elucidate the roles of U2AF1 in the DNA damage response in non-transformed cells in three parts. Part 1: To better model the S34F mutation, we engineered human retinal pigment epithelial (RPE-1) cells to express the S34F heterozygous or homozygous mutation conditionally. We found that cells expressing heterozygous and homozygous S34F mutation exhibited reduced proliferation, potentially due to p53-mediated cell cycle arrest. Ultimately, we will use this cellular model to identify the genetic vulnerabilities of U2AF1 S34F cells treated with poly(ADP-ribose) polymerase 1 (PARP) inhibitors by CRISPR/Cas9 genetic screens, which have been shown to selectively kill S34F mutants. Part 2: To explore the functions of U2AF1 S59 phosphorylation, we will express WT-, phospho-dead or phospho-mimetic versions of U2AF1 while knocking out endogenous U2AF1 in cells. This will enable us to investigate the contribution of U2AF1 phosphorylation in response to DNA damage agents. Part 3: U2AF1 is alternatively spliced, generating two isoforms: a and b. Our preliminary data suggested that both isoforms are required for maintaining cell growth. We hypothesize that the two isoforms may play different roles in DDR. To test this hypothesis, we will generate isoform-specific cell lines and evaluate their fitness in responding to DNA damage. Overall, this project will provide insights into whether the alternative splicing mediated by U2AF1 contributes to genome maintenance.