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POSTER ABSTRACTS

603.LYMPHOID ONCOGENESIS: BASIC

Hnrnpu mutations Are Haploinsufficient and Alter the Transcriptome of MYC-Driven Lymphomas

Qurat Ul Ain Qureshi, BSc[1], *Krysta Mila Coyle, PhD*[1], *Nicole Thomas*[1], *Brett Collinge*[2], *Kostiantyn Dreval*[3], *Laura Hilton, PhD*[3], *Jasper Wong*[4], *David W. Scott, PhDMD,FRACP,FRCPA*[5,6], *Timothy Audas, PhD*[1], *Ryan Morin, PhD*[3,1]

[1] Simon Fraser University, Burnaby, Canada
[2] BC Cancer, Vancouver, CAN
[3] Genome Sciences Center, BC Cancer Research Institute, Vancouver, Canada
[4] University of British Columbia, Vancouver, CAN
[5] Department of Medicine, University of British Columbia, Vancouver, Canada
[6] Centre for Lymphoid Cancer, BC Cancer Research Centre, Vancouver, Canada

Introduction:
Burkitt lymphoma (BL) is an aggressive B-cell cancer, characterized by translocations that juxtapose a potent immunoglobulin enhancer with the *MYC* oncogene. *MYC* translocations are also found in 10% of tumors with diffuse large B-cell lymphoma morphology. When a *BCL2* translocation is also present, these high-grade B-cell lymphomas (HGBCL-DH- *BCL2*) have poor prognosis. While sustained *MYC* expression can promote cell activation and proliferation, it also has potent and acute effects on programmed cell death, which cancer cells must overcome in order to survive. Identification and functional characterization of common mutations that cooperate with MYC are thus important for understanding its role in pathobiology.
Methods:
Patient samples were obtained from an ongoing meta-analysis of mature B-cell neoplasms including unpublished data from the Lymphoma/Leukemia Molecular Profiling Project. Simple somatic mutations were identified from whole genome or exome sequencing data using an ensemble of variant callers. *MYC* rearrangements breakpoints were identified using GRIDSS and Manta. All functional studies were performed in an EBV+ BL cell line (Raji). *HNRNPU* mutations were introduced into cells using IDTs ALT-R CRISPR-Cas9 system, clonally isolated, and verified by sequencing. Subsequent RNA-sequencing was performed. Knockdown and overexpression experiments were performed using an *HNRNPU* expression vector or siRNA targeting endogenous *HNRNPU*. *HNRNPU* eCLIP in HepG2 and K562 cells were obtained from the ENCODE project consortium. Differential gene expression was analysed with DESeq2. Peak-calling and individual crosslink sites were detected using PureCLIP.
Results:
Within the sequencing data, we noted that several RNA binding proteins were commonly mutated in BL and HGBCL-DH-*BCL2*. Mutations affecting *HNRNPU* were identified in tumors from 11.6% of HGBL-DH- *BCL2* and 5.6% of BL patients. These mutations were predicted to be inactivating but, unlike conventional tumor suppressor genes, an inactivating mutation on the other allele was never observed. *HNRNPU* mutations were significantly enriched in EBV+ BLs. The gene product (hnRNP U) is an RNA- and DNA-binding protein that plays a central role in gene expression regulation.
To understand their biological effect in aggressive lymphomas, we introduced heterozygous inactivating mutations in the most affected region of *HNRNPU*. Clones with confirmed knockout exhibited reduced expression at the mRNA and protein level. Through gene expression analysis, comparing the parental to two mutant lines, we identified 615 differentially expressed genes (358 downregulated, 219 upregulated). Pathway enrichment analysis revealed reduced expression of genes in several relevant pathways such as MYC, TP53 and DNA damage response. This was consistent with our observation of reduced MYC protein in the mutant lines. A role of hnRNPU-in MYC modulation was further in experiments when *HNRNPU* knockdown reduced and overexpression increased *MYC* expression. Overexpression of hnRNP U also resulted in cellular stress and a decrease in cell proliferation.
Using actinomycin D chase experiments, we found that hnRNPU loss leads to reduced *MYC* transcript stability. Crosslinking immunoprecipitation suggests that hnRNP U binds directly to the *MYC* transcript in poly G tracts in intron 1. This region is predicted to fold into G-quadruplexes, a secondary structure that can be bound by hnRNP U. *HNRNPU* mutations are only

observed in tumors with an intact MYC locus rather than those with a rearrangement in intron 1. Taken together, these findings suggest that the region of *MYC* upstream of intron 1 may be relevant for *HNRNPU*-mediated modulation of MYC.
Conclusion:
HNRNPU mutations are novel recurrent driver mutations specifically within *MYC* translocated B-cell lymphomas. *HNRNPU* acts as a modulator of MYC expression, and the most common mutations are predicted to negatively impact this role. We propose a model where *HNRNPU* mutations moderate *MYC* expression, thus buffering MYC-induced apoptosis and proteotoxic stress. Further experiments to explore the role of *HNRNPU* binding on *MYC* expression and the potential role in buffering the proteotoxic stress associated with *MYC* translocations are ongoing. Insights into the mechanisms contributing to disease will support ongoing work to establish in vitro and in vivo models for future therapeutic investigations.

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