00395 Synergistic Lesions in Diffuse Large B-cell Lymphoma

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Aims: Diffuse large B - cell lymphoma (DLBCL) is the most common aggressive lymphoma among adults. About half of the patients can be cured with intensive chemotherapy treatment called R - CHOP with 4 drugs named cyclophosphamide, doxorubicin, vincristine, and prednisone coupled with monoclonal antibody rituximab. However the toxicity of R - CHOP and the heterogeneous nature of the disease call for a need to identify a novel therapeutic target. Human DDX3X is an X - linked DEAD box RNA helicase which is frequently mutated in Burkitt Lymphoma (BL) and NK/T - cell lymphoma. In this study, we aimed to understand the genetics of DDX3X knockdown and its effect on chemotherapy resistance in DLBCL.

Methodology: Generation of customized antibody specifically targeting DDX3X and not its Y - linked homologue DDX3Y to confirm knockdown through western blot in DLBCL and BL cells with DDX3X short hairpin RNA (shRNA) gene knockout. MTS cell proliferation assay was performed to investigate chemotherapy resistance in DDX3X depleted cells against doxorubicin. The loss of function of DDX3X on the expression of Cyclin D1 was analyzed by western blot. Whole exome sequencing of 9 Relapse/Refractory (R/R) - DLBCL biopsies from Singapore was performed.

Result: Specificity of the customized affinity - purified DDX3X antibody was confirmed by the presence of a defined band of interest and the absence of detection in DDX3X and DDX3Y - transfected HEK 293T cells respectively. Doxorubicin resistance was seen in shDDX3X1 and shDDX3X2 hairpins, in DLBCL - derived U2932 and BL - derived BJAB cells with an increase in half maximal inhibitory concentration (IC50) as compared to controls. Increase in cyclin D1 expression was seen in DDX3X - depleted cells. Sequencing analysis found somatic DDX3X mutation in 4 out of 9 of the cases.

Conclusion: Our results underlined the potential driver role of DDX3X downregulation to the pathogenesis of DLBCL with DDX3X being the most frequently mutated gene in the R/R - DLBCL cohort and is associated with doxorubicin resistance in vitro.