DDX3X Loss Is An Adverse Prognostic Marker In Diffuse Large B-Cell Lymphoma And Is Associated With Chemoresistance In Aggressive Non-Hodgkin Lymphoma Subtypes

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Introduction

- Non-Hodgkin lymphoma (NHL) subtypes, encompassing common diffuse large B-cell lymphomas (DLBCL), often display chemoresistance to treatment
- DEAD box helicase 3, X-linked (DDX3X) is an ATP dependent RNA helicase involved in different cellular processes including transcription and signal transduction
- DDX3X displays variable suppressive or oncogenic roles in different types of tumour. However, the role of DDX3X in NHL subtypes remains unclear.
- This study investigates the effect of DDX3X mutation/loss on NHL subtypes including diffuse large B cell lymphoma (DLBCL), Burkitt lymphoma (BL), cutaneous T cell lymphoma (CTCL), and NK/T-cell lymphoma (NKTCL)

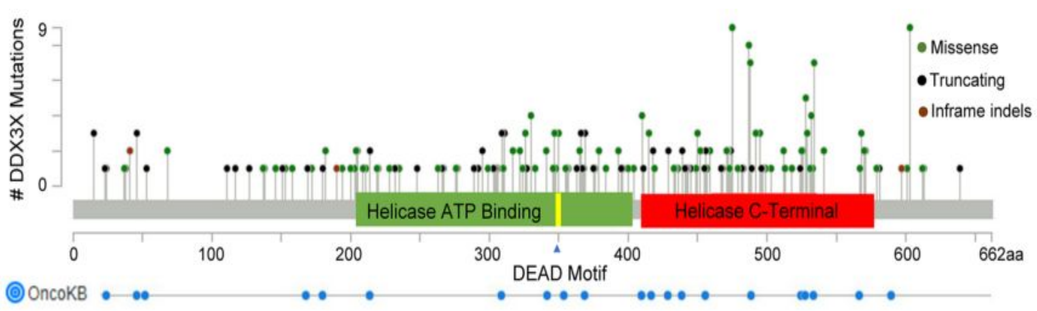
Methods

- NHL cell lines U2392 and BJAB (DLBCL), Raji (BL), SNK1, SNK2, NKYS (NKTCL), HuT78, MJ, MyLa (CTCL)
- CRISPR/Cas9 technique to introduce DDX3X knock-in mutation
- Small interfering RNA (siRNA) and short hairpin RNA (shRNA) technique to knock-down DDX3X in NHL cells
- MTS assay to quantify cell viability and proliferation
- Checkerboard assay to determine drug synergism
- RNA sequencing and gene analysis (IPA, DAVID, GSEA)
- Quantitative reverse transcription-polymerase chain reaction (RT-qPCR) to quantify mRNA level
- Western immunoblot analysis to quantify protein levels
- Real time impedance-based Matrigel assay to quantify cell invasiveness
- Xenograft of U2392 cells with mutant DDX3X-R475C was done into mice

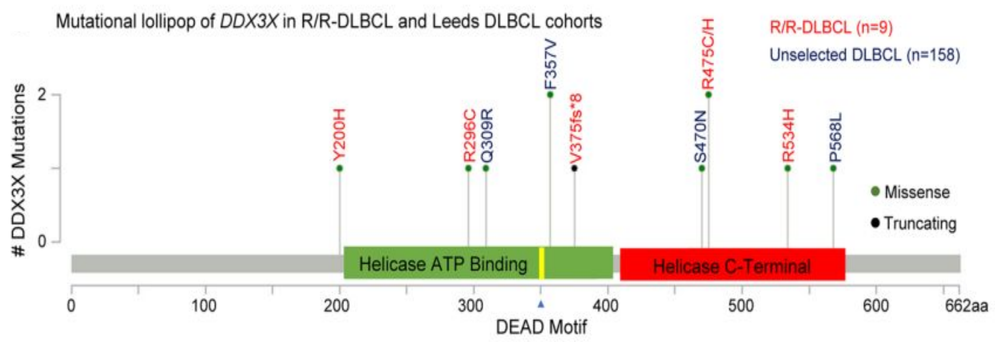
Results

1. Lollipop Plots showing DDX3X mutations in NHL (a) and DLBCL patients (b)

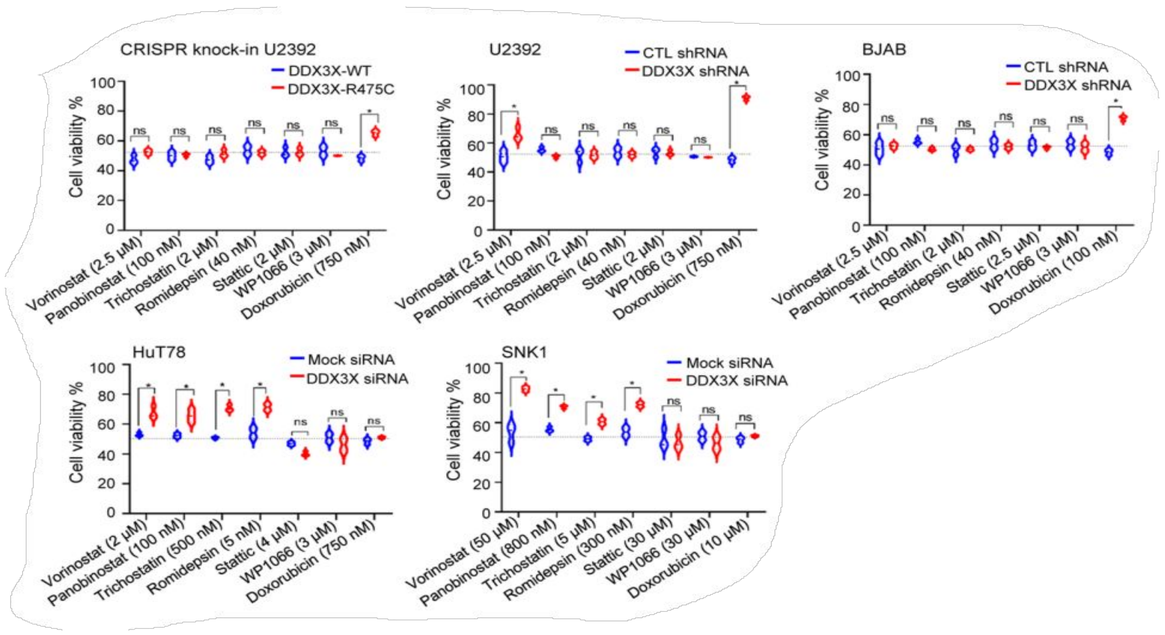
(a)



(b)



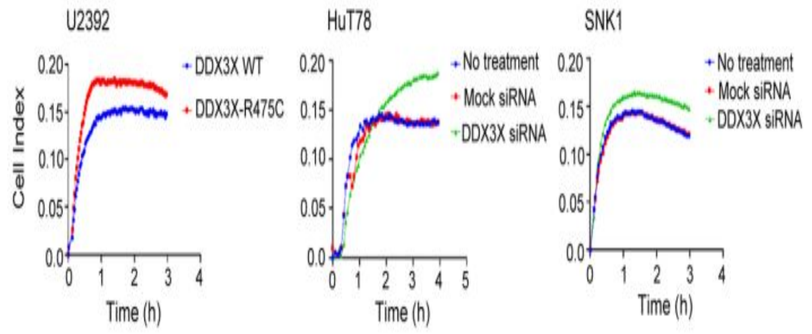
2. Cell viability assay shows that NHL cell lines with DDX3X mutation/loss are sensitive to STAT3 inhibitors



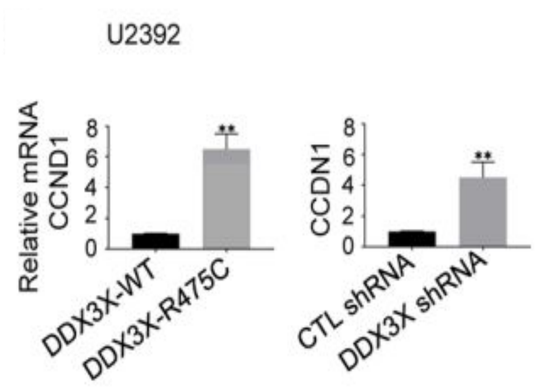
3. Checkerboard Assay shows true chemoresistance in NHL mutant subtypes

NHL cell subtypes	Combination Index
U2392 DDX3X-WT	1.88804
U2392 DDX3X-R475C	1.63570
U2392 CTL shRNA	1.24540
U2392 DDX3X shRNA	1.75190
HuT78 Mock siRNA	1.05000
HuT78 DDX3X siRNA	0.75812
SNK1 Mock siRNA	1.32272
SNK1 DDX3X siRNA	1.13760

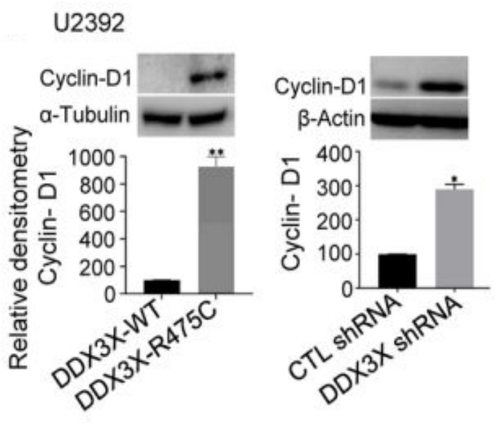
4. Matrigel analysis shows increased migration of NHL cell lines with DDX3X mutation/loss



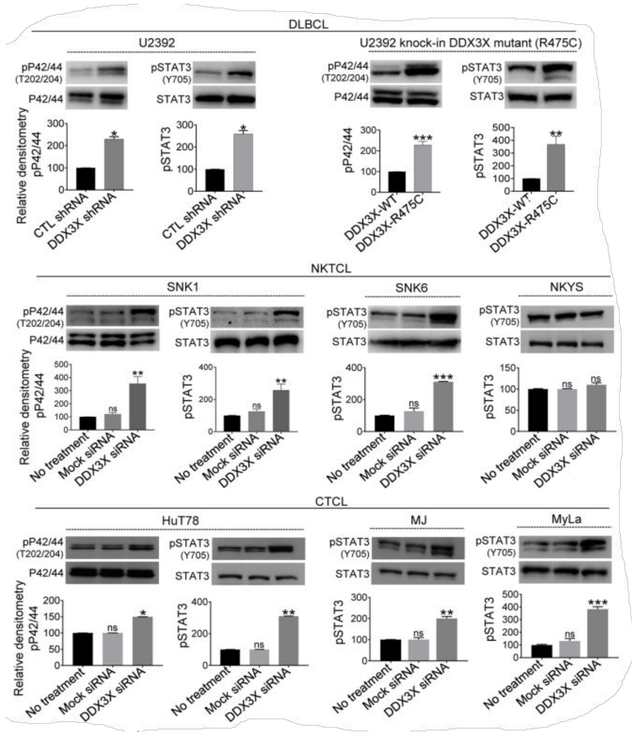
5. RT-qPCR shows cyclin-D1 overexpression in DDX3X mutant/depleted DLBCL cells



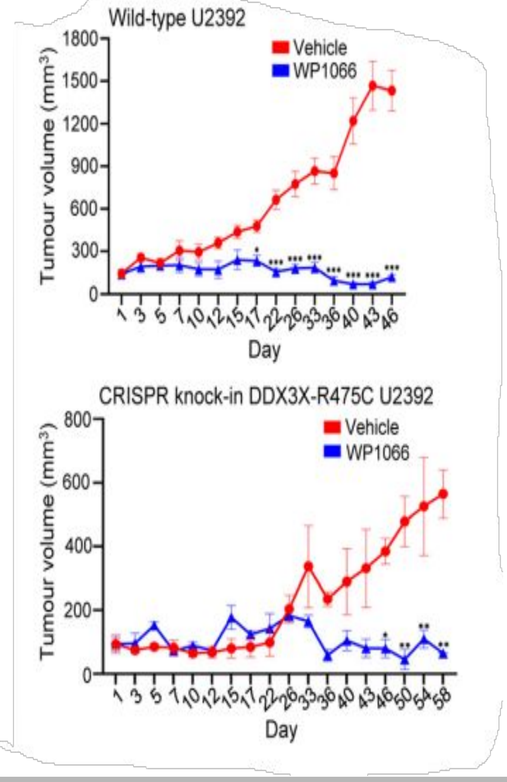
6. Western Immunoblot analysis further confirms cyclin-D1 protein upregulation in DDX3X mutant/depleted DLBCL cells



7. Western Immunoblots show increased phosphorylation levels of STAT3/MAPK in DDX3X-mutant/depleted DLBCL, NKTCL, and CTCL cells



8. In vivo tumour xenograft studies with mice show that DDX3X-mutant DLBCL tumours are sensitive to STAT3 inhibition



Conclusion, Clinical implications, Perspectives

- The mutation/loss of DDX3X enhances the proliferative and migratory potential of NHL cell subtypes (DLBCL, NKTCL, CTCL)
- The mutations/loss of DDX3X enhances the activation of oncogenes STAT3/MAPK, by the increase of phosphorylation
- DDX3X mutations/loss are associated with worse clinical outcomes in DLBCL, and are implicated with chemoresistance in NHL subtypes
- Targeting the STAT3 pathway might be a better approach to treat chemoresistant NHL (DLBCL, NKTCL, CTCL) patients with mutated DDX3X
- Currently, STAT3 inhibitor AZD9150 is in phase 1b trial in a subset of patients with heavily pretreated lymphoma
- Further studies are required to improve understanding over DDX3X mutation/loss, which could possibly improve the risk stratification of aggressive NHL subtypes
- Having a greater understanding on the implication of DDX3X could also open up further studies into more advanced therapeutic options for patients with poorer prognosis

Reference: Kizhakeyil A, Zaini NBM, Poh ZS, Wong BHS, Loh X, Ng AS, Low ZS, Prasannan P, Gong C, Tan MGK, Nagarajan C, Huang D, Lu PW, Lim JQ, Barrans S, Ong CK, Lim ST, Chng WJ, Follows G, Hodson DJ, Du MQ, Goh YT, Tan SH, Grigoropoulos NF, Verma NK. DDX3X loss is an adverse prognostic marker in diffuse large B-cell lymphoma and is associated with chemoresistance in aggressive non-Hodgkin lymphoma subtypes. Mol Cancer. 2021 Oct 16;20(1);134