DDX3X loss is an adverse prognostic marker in diffuse large B-cell lymphoma and is associated with chemoresistance in aggressive non-Hodgkin lymphoma subtypes[1]

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BACKGROUND

DEAD box helicase 3, X-linked (DDX3X) is an ATP-dependent RNA helicase proposed to have genetic links to the aggressiveness of Non-Hodgkin lymphoma (NHL) but its role in chemoresistant NHL patients remains debatable. However, if found in genetic workups of NHL patients, it provides opportunities to start early initiation of effective treatment. Thus, genetic testing for DDX3X mutations prior to therapy may benefit patient outcomes.

In this paper, DDX3X mutations were demonstrated to be associated with poor prognosis in DLBCL by increasing STAT3/p42/p44 phosphorylation.

This research study aimed to find the role of DDX3X in aggressive NHL subtype DLBCL and the development of chemoresistance.

METHODS

Sample

DLBCL cases with mutated DDX3X obtained from cBioPortal (49/1343), ICGC (63/1319), COSMIC (160/5160) portals

Identified 165 missense, 81 truncating, 3 in-frame indel mutation variants in DDX3X in NHL subtypes

Subtyping DDX3X mutations

1) Accuracy of DDX3X as prognostic marker in DLBCL was assessed by correlation of medial survival rates.
2) Whole exome sequencing identified damaging DDX3X mutations.

DDX3X mutation in drug resistance

1) CRISPR knock-in technique expressed mutant DDX3X R475C in U2392 cells
2) Cells treated with histone deacetylase (HDAC) inhibitors/STAT3 inhibitors /doxorubicin
3) MTS based assay analysed cell viability

DDX3X mutations effect on proliferation and migratory potential of NHL cell subtypes

1) Real time monitoring of cells transmigrating through Matrigel using impedance-based measurement (determining invasive potential)
2) Western immunoblot analysis done on proteins and detected increased vimentin expression

Effect of DDX3X mutation in NHL subtyped derived cells on cellular pathways

Gene network analysis studying effect of DDX3X mutation on cyclin-D1 and JAK-STAT3 pathways in NHL cells
1) RT-qPCR and Western immunoblot analysis used to verify cyclin-D1 mRNA and protein levels
2) Western immunoblot used to determine STAT3/ MAPK activation

RESULTS

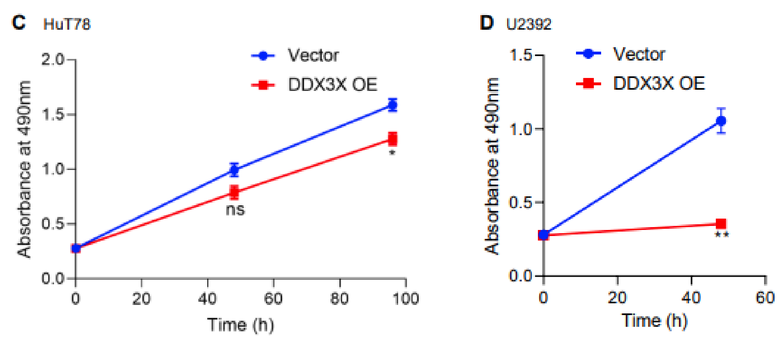

Figure 1. . Effect of DDX3X overexpression on the rate of proliferation of CTCL and DLBCL cells. Rates of mutant cell line proliferation were measured after being nucleofected with vector or wild type DDX3X.

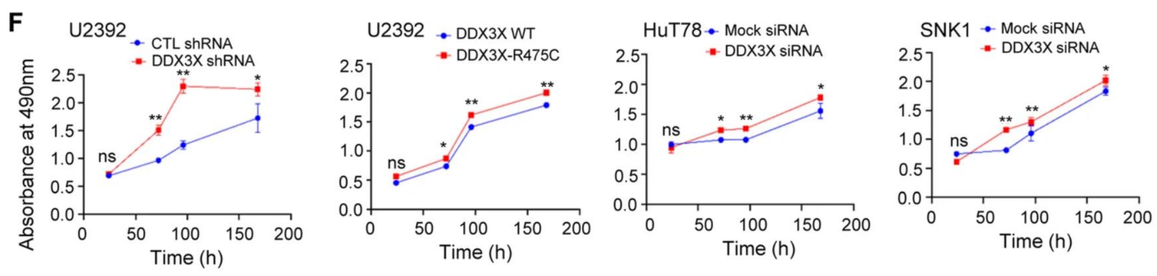

Figure 2. The effect of DDX3X mutation/knockdown on cell proliferation rates in mutant cell lines at different time points up to one week.

DDX3X mutation/loss in NHL cell lines enhanced proliferation and invasiveness

DDX3X mutated/depleted cell lineages showed significant increase in proliferation rates (Fig. 1,2). Mutant cell lines showed significantly increased invasiveness of mutated cells and increased vimentin expression, associated with aggressive transformation in lymphoma.

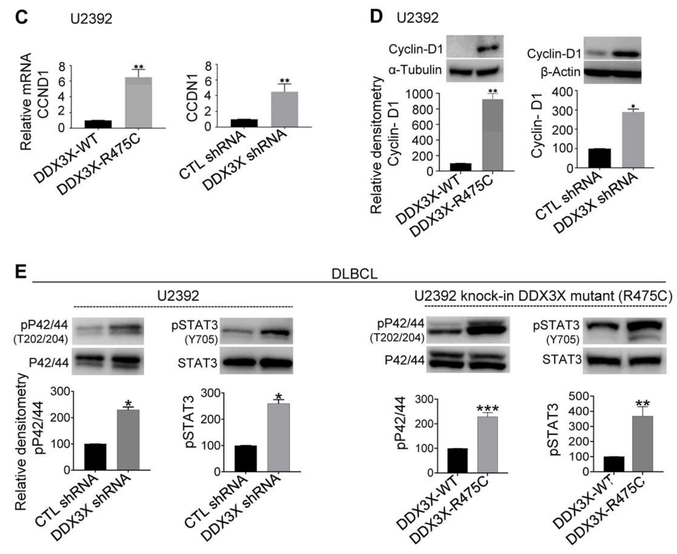

Fig 3. C. mRNA levels of cyclin-D1 (CCND1) in both control and mutant cell lines. D. CCND1 expression levels in both control and mutant cell lines quantified by Western Immunoblotting. E. Phosphorylation levels of STAT3 and p42/44 in control and mutant cell lineages.

DDX3X mutations/loss associated with increase in cyclin D1 expression

There was a demonstrated upregulation of cyclin-D1 and JAK-STAT3 pathways, with significant elevation of cyclin-D1 specifically (Fig. 3C, D), possibly related to chemoresistance in various cancers.

Enhanced STAT3/MAPK activation downstream of DDX3X

Increased phosphorylated STAT3 levels and p42/22 MAPK pathways were found in mutated NHL cells. Yet, STAT3 knockdown had no effect on DDX3X levels, suggesting its hyperphosphorylation as a downstream effect of DDX3X mutation. Hence, these pathways are likely mediators of tumorigenic effects in DDX3X mutation.

DISCUSSION

Findings suggest DDX3X mutated cells are sensitive to STAT3 inhibition due to its downstream effects of hyperphosphorylation of the STAT3/p42/p44 pathway. This potentially supports the role of genetic testing in NHL patients as presence of DDX3X mutations can guide chemotherapeutic modalities by encouraging use of STAT3 inhibitors.

Although there is increased understanding on the role of DDX3X in cancer cells now, controversy on its exact function on cellular processes remains [2]. Hence, further research is required to test sensitivity of DDX3X mutated cells in other NHL cell types to STAT3, HDAC, and other inhibitors not used in this study.

CONCLUSION

DDX3X mutations in DLBCL contribute to the aggressiveness of the disease and chemoresistance faced by patients. DDX3X mutations are shown to have downstream effects on the STAT3 pathway, suggesting sensitivity to STAT3 inhibition, hence providing an avenue of therapy for chemoresistant NHL.

REFERENCES

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ACKNOWLEDGEMENTS

The authors of this poster would like to sincerely thank Prof Navin for his guidance through the paper and designing of the poster.

The authors of this paper would like to acknowledge Dr. George Vassiliou and Dr. Niccolo Bolli, Wellcome Sanger Institute, Wellcome Genome Campus, Hinxton, UK for assistance with WES. They would also like to thank Professor Andrew Jack, HMDS, Leeds Cancer Centre for providing DNA samples from DLBCL cases.