Reducing autophagy potentiates the restoration of MHC-I genes in Germinal B-cell Diffuse Large B-cell lymphomas

Abstract ID:439

Category: Clinical hemato-oncology

Presented at SOHC 2024 from 20 – 22 November 2024

Harpreet Mandhair[1,2], Yara Banz[3], Joern Dengjal[4], Mario P. Tschan[3] and Urban Novak[1,2]

[1]Department of BioMedical Research, University of Bern, Bern, Switzerland, [2]Department of Medical Oncology, Inselspital, Bern University Hospital, University of Bern, Bern, Switzerland, [3]Institute [4]Department of Biology, University of Freiburg, Freiburg, Switzerland

INTRODUCTION

Diffuse Large B-cell Lymphoma (DLBCL) is an aggressive mature B-cell malignancy, characterised by significant clinical and biological heterogeneity. The two major DLBCL subtypes: germinal B-cell (GCB) and activated B-cell (ABC) lymphomas. Immune evasion is a major obstacle for DLBCL treatment. Major histocompatibility complex I (MHC-I) is largely compromised in GCB tumours. Genetic aberrations in the antigen presentation machinery HLA and β2M genes contributes to MHC-I deficiency reducing the tumour immunogenicity. The exact mechanisms underlying the loss of MHC-I remains elusive. Macroautophagy (herein autophagy) is a major lysosome-dependent degrading pathway. Autophagy initiation activates the ULK1 complex. Deregulated autophagy rewires metabolism and confers resistance by regulating immune cells recruitment in the tumour microenvironment, decreasing the tumour immunosurveillance. In solid cancer, autophagy is reported to degrade MHC-I to promote immune evasion. We speculate that autophagy inhibition will restore surface MHC-I.

OBJECTIVES

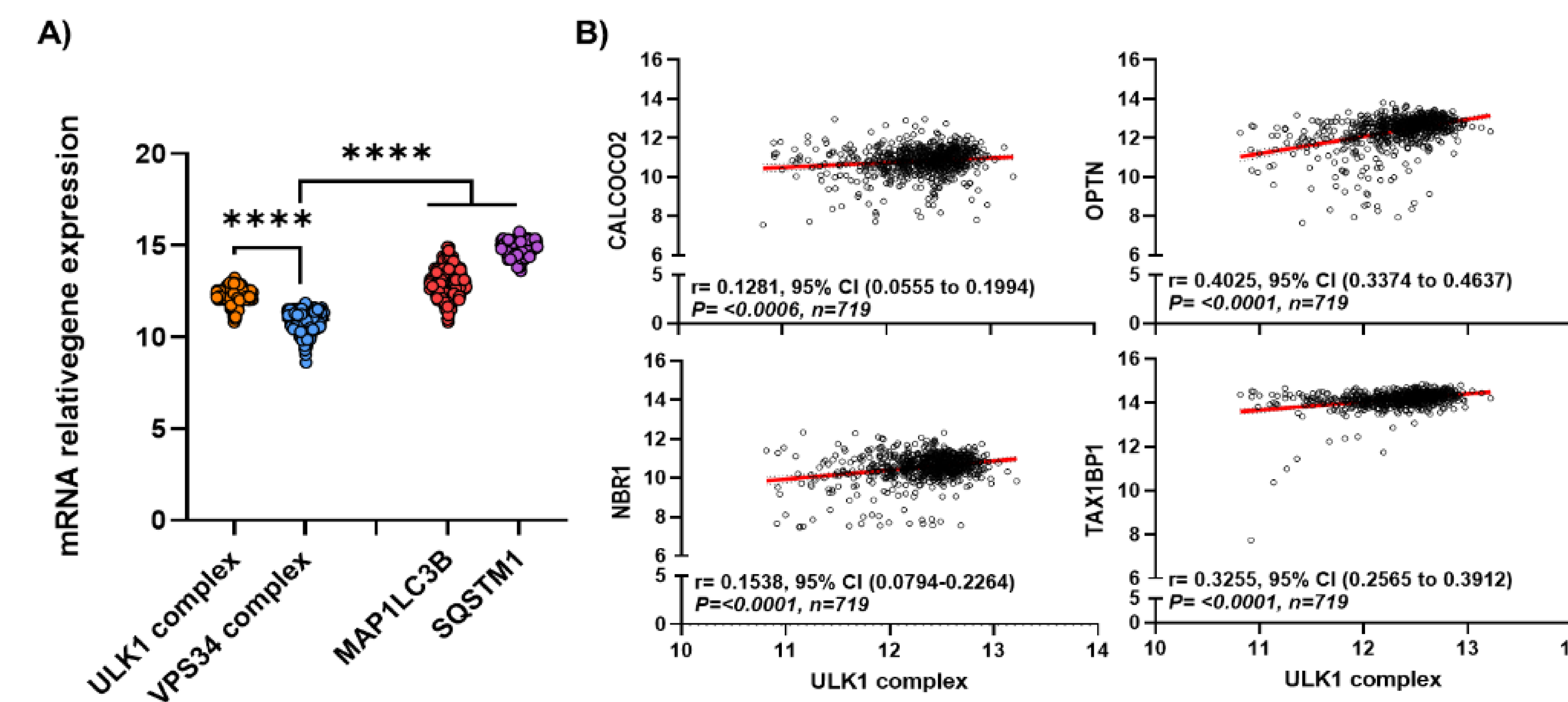
The overarching aim of the study is to investigate the role of autophagy in regulating MHC-I expression in GCB DLBCL and to explore whether autophagy blockade can restore MHC-I levels. Ultimately by enhancing the immunogenicity of DLBCL tumours, potentially improving their responsiveness to immune therapies.

METHODS

- Gene-expression profiling of GCB patients from (NCT01324596) was obtained. Correlations using Spearman rank was used to correlate autophagy related genes with antigen presenting genes. Statistics were performed using GraphPad Prism 9. P valves less than 0.05 were considered significant.
- RNA sequencing was undertaken on Oci-Ly1 (GCB) and Oci-Ly3 (ABC) cell lines treated with MRT68921 (2.5µM. t= 60min) vs. Vehicle. Gene Set Enrichment (GSEA) was performed using GSEA software v.4 (Broad Institute, Cambridge). Gene ontology analysis was evaluated for all relevant pathway genes obtained from Path cards database (pathcards.genecards.org).
- Liquid chromatography- mass spectrometry/ mass spectrometry (LC-MS/MS) was to identify and quantify proteins in ABC Oci-Ly1, TMD8, U2932 and GCB Oci-Ly1 and Oci-Ly18 cell lines.

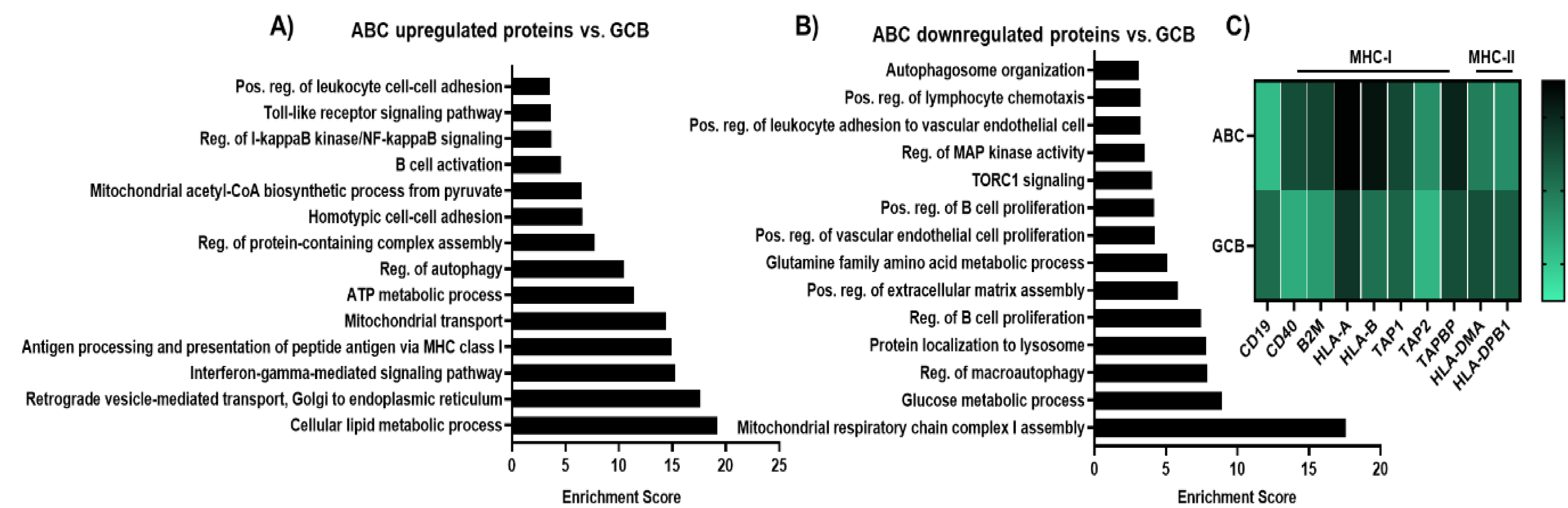
RESULTS

Figure 1. DLBCL patients significantly express pro-autophagic genes.



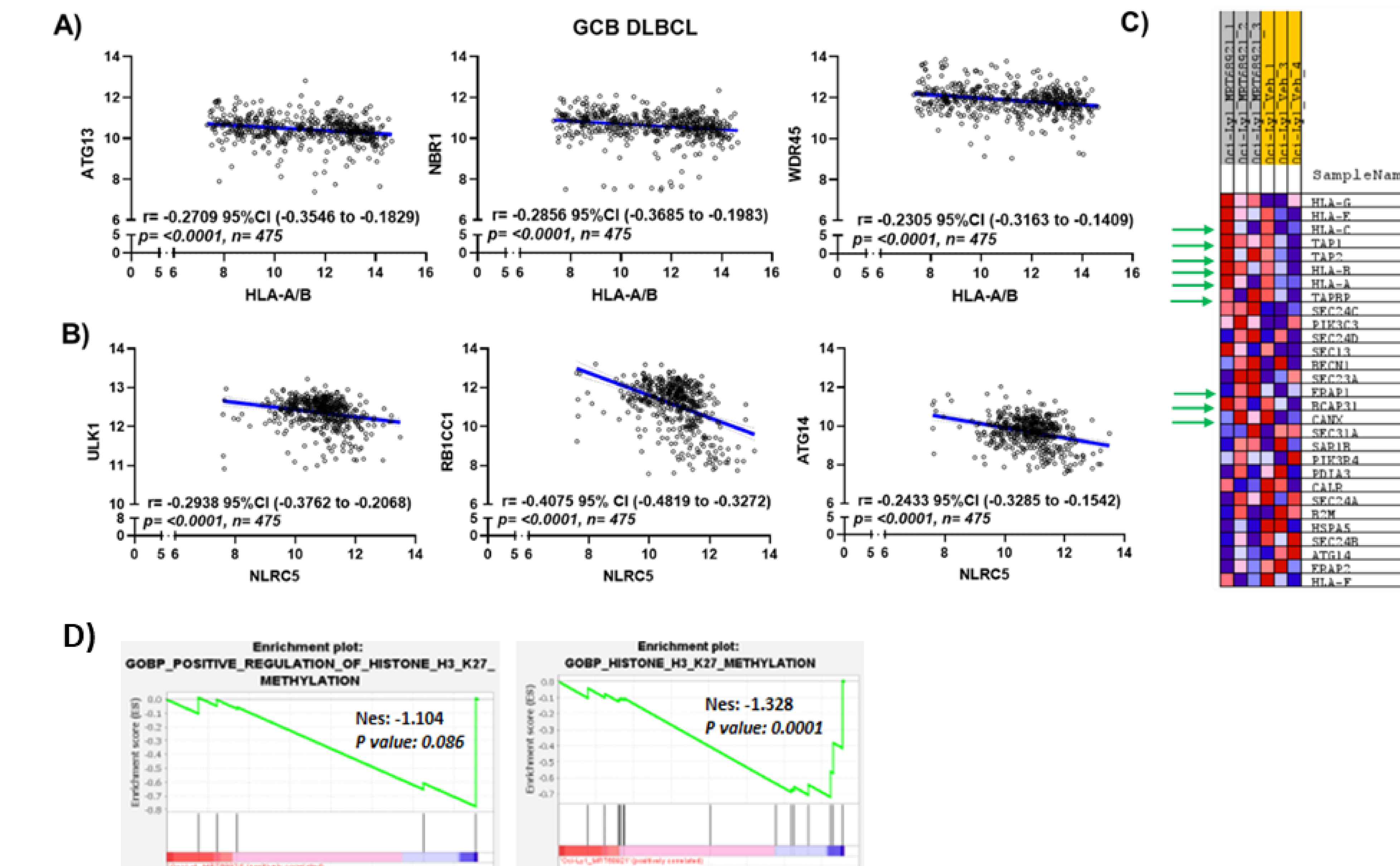
A) Genes associated with the ULK1 complex and autophagosome cargo receptor genes *MAP1LC3B/* LC3B and *SQSTM1* were markedly expressed compared to the VPS34 complex in DLBCL tumours *(n=719)*. **B)** Spearman's rank correlation coefficient determined that multiple cargo receptors positively correlated with ULK1 complex in DLBCL patients *(n=719)*.

Figure 2: Differential protein expression of autophagy, adhesion markers and MHC-I in DLBCL subtypes.



LC-MS/MS was used to evaluate the total proteome of ABC and GCB cell lines and compare the differentially expressed proteins. Gene Ontology was used to demonstrate the enrichment of proteins **A)** comparing the upregulation of proteins in ABC compared to GCB and **B)** downregulation of ABC proteins compare to GCB. **C)** Heatmap illustrates the protein expressions of MHC molecules in DLBCL subtypes

Figure 3: The autophagic pathway negatively correlated with MHC molecules in DLBCL subtypes.

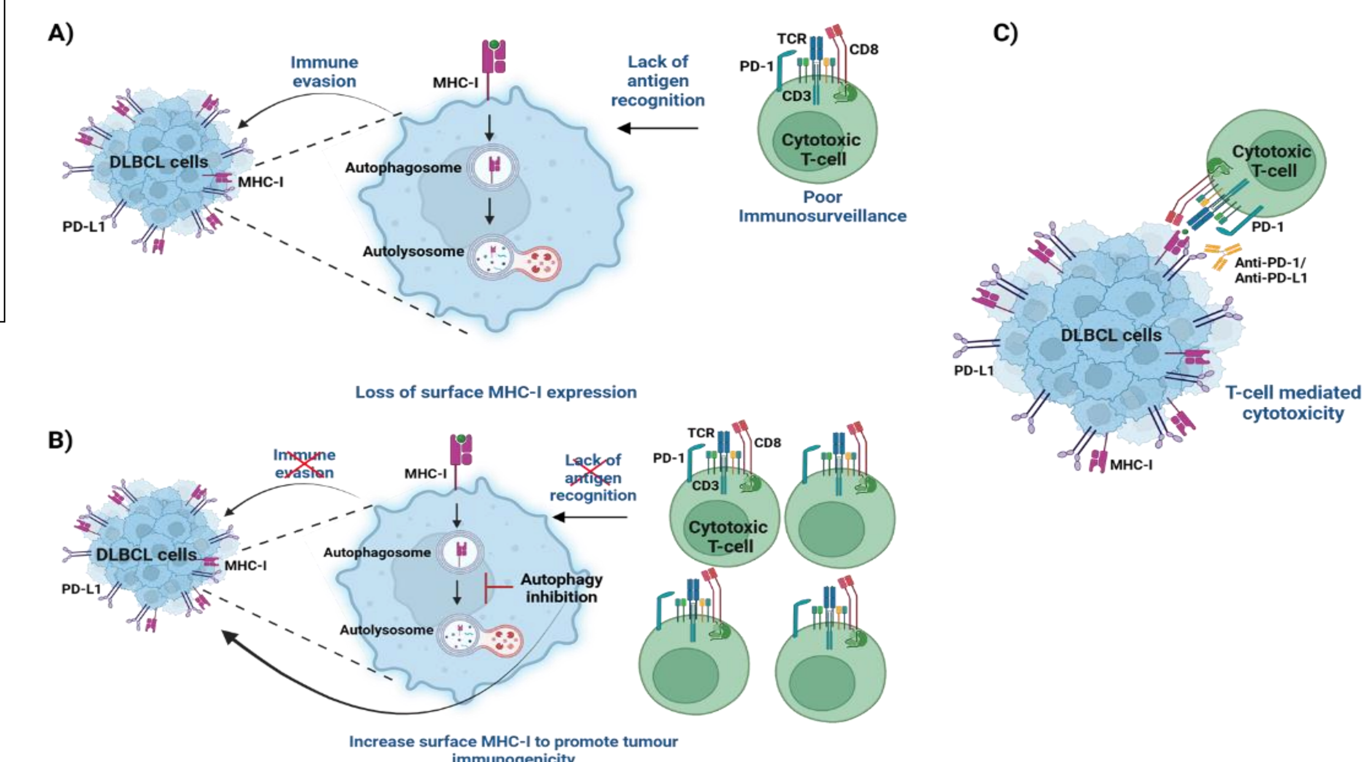


A-B) Spearman's rank correlation coefficient determined the association between multiple autophagy related genes and MHC-I molecules *HLA-A/B* and transactivator in GCB patients (*n=475*). **C)** RNA-sequencing was performed on GCB Oci-Ly1 cells treated with MRT68921 vs. vehicle for 1 h. Positive regulators of MHC-I transcripts were increased (as indicated by green arrows). **D)** Gene-set enrichment analysis determined that ULK1 inhibition significantly decreased transcripts of histone 3 methylation. This is relevant as *EZH2* is a regulator of H3K27me and transcriptionally regulates *NLRC5.*

Conclusion

Our data provides a rationale to target ULK1-mediated autophagy in GCB DLBCL. Autophagy inhibition potentiates the restoration of MHC-I surface levels and elevate tumour immunogenicity to enhance the antigen presentation to T-cells.

Figure 4: Graphical summary



A) Somatic mutations in DLBCL and commitment increased autophagy degrades the surface MHC-I expression to promote immune evasion and reduce the tumour immunogenicity. **B)** Autophagy blockade enriches the total and surface MHC-I expression allow cytotoxic T-cells to recognise the tumour cells. **C)** Increased MHC-I-mediated by autophagy inhibition would synergise with anti-PD-1 or L1 therapy to elevate therapy response.

Contact: Urban.Novak@insel.ch; Harpreet.Mandhair@unibe.ch