Identification of novel targets that upregulate CD20 expression in rituximab-resistant cells with the use of genome-wide CRISPR screening

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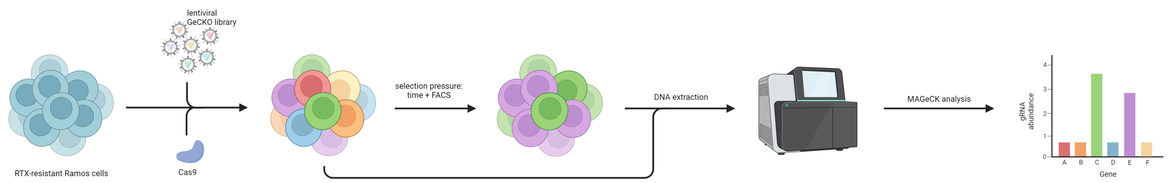
Introduction & Aims

Despite progress in the therapy of B-cell malignancies, administration of anti-CD20 monoclonal antibodies (mAb) like rituximab (RTX) remains the first-line therapy in most cases. However, malignant B cells often downregulate the expression of CD20 on their surface, resulting in mAb resistance and subsequently in therapy failure. By modulating CD20 levels it may be possible to **enhance anti-CD20 mAbs efficacy** and to reverse the progression of the disease. Therefore, it is crucial to identify the molecular mechanisms underlying the **regulation of CD20 expression**. Our aim was to perform a **genome-wide CRISPR/Cas9 screening in RTX-resistant cell line** to identify genes that regulate the surface expression of CD20 and validate genes identified by the screening.

Methods



CRISPR/Cas9 Screening

The RTX-resistant CD20^{low} cell line (which was previously established in our laboratory) was transduced by a genome-wide CRISPR/Cas9 knockout library (GeCKO v2) to obtain population of single-gene knockouts. After 2.5-week cultivation, the top 5% of cells with the highest CD20 expression were sorted out using fluorescence-activated cell sorting. Using next-generation sequencing in combination with bioinformatical tool MAGeCK, we identified gene knockouts that were significantly enriched in the sorted population.

Validation of screning hits:

New gRNAs were designed for selected top hits identified by screening and used to produce lentiviral particles. RTX-resistant Ramos cells were transduced by these lentiviral particles to produce single-gene knockout population. Single-cell clones were isolated. Levels of CD20 were observed using flow cytometry and western blotting. To assess rituximab cytotoxicity, Ramos cells were incubated with the RTX and human serum (as a source of complement) for 72 hours and then viability was measured by cell titer glo assay.

Results

Figure 1. CRISPR/Cas9 screen reveals several promising hits
The screening revealed several genes whose disruption led to the upregulation of CD20 surface expression. As top hits were considered genes with at least three out of six gRNAs significantly enriched and with false discovery ratio below 0.05.

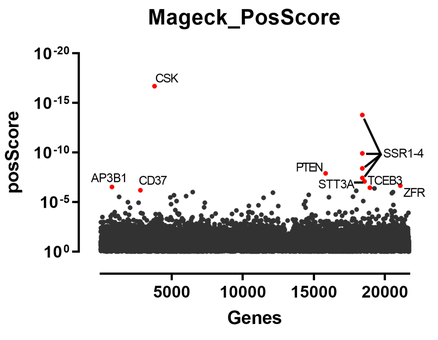


Figure 2. CSK validation
CSK codes a negative regulator of Src family kinases. Ramos RTXr CSK^{KO} cells have higher expression of CD20 on their surface as measured by flow cytometry and western blot detected higher total amount of CD20 protein in cells.

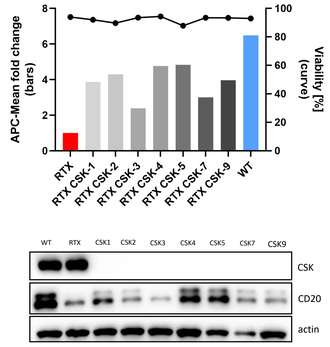


Figure 3. Response to rituximab in CSK^{KO} cells
The disruption of *CSK* in Ramos RTX-resistant cells restores their sensitivity to rituximab to the degree of original WT cells. The cells were incubated with RTX in presence of human serum and viability was assesed by cell titer glo assay.

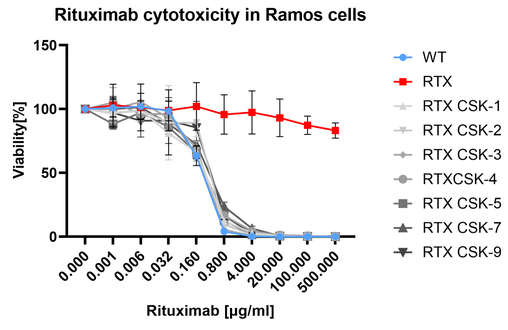


Figure 4. SSR1 validation
SSR1 codes α subunit of translocol-associated protein (TRAP) complex in endoplasmic reticulum. The complex is involved in several ER processes. Ramos RTXr SSR1^{KO} cells have higher expression of CD20 on their surface as measured by flow cytometry and western blot detected higher total amount of CD20 protein in cells.

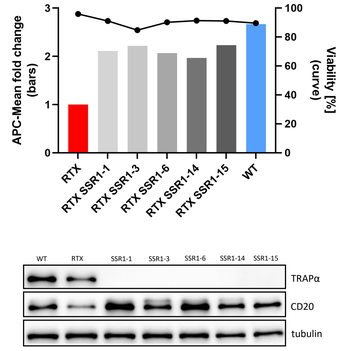


Figure 5. Response to rituximab in SSR1^{KO} cells
The disruption of *SSR1* in Ramos RTX-resistant cells restores their sensitivity to rituximab to the degree of original WT cells. The cells were incubated with RTX in presence of human serum and viability was assesed by cell titer glo assay.

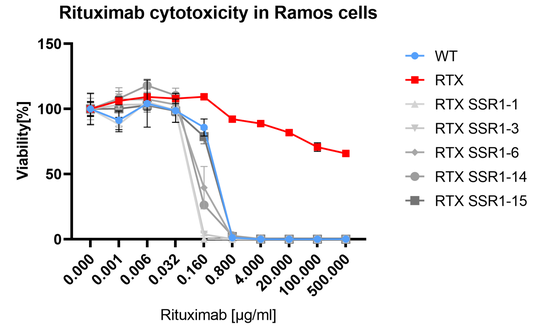


Figure 7. CD37 validation
CD37 codes glycosylated membrane protein expressed on B cells. Its function is not well described. Knock-out of *CD37* in Ramos RTXr cells leads (A) to the increase in CD20 expression on the surface of cells as assesed by flow cytometry. CD37 disruption in wild-type Ramos cells (B) did not upregulate CD20 expression.

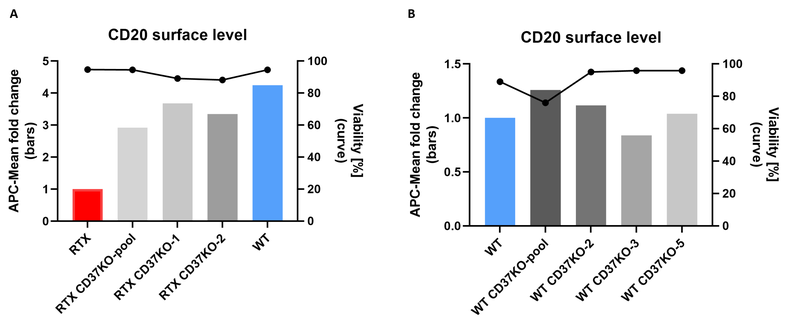
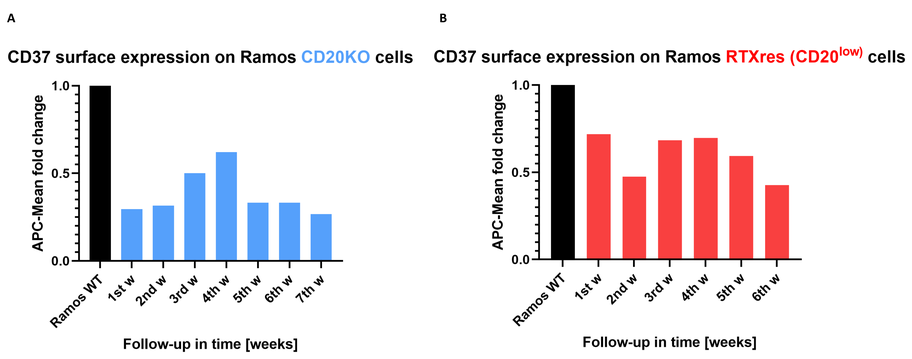


Figure 8. CD37 expression in CD20^{low} cell lines
Expression of CD37 on the surface of Ramos CD20^{KO} cells (A) and Ramos RTX-resistant cells (B) was measured by flow cytometry. Both cell lines with lower CD20 suprisingly express lower levels of CD37 on their surface.



Conclusion

CRISPR/Cas9 screening revealed several genes whose disruption led to upregulation of CD20 surface expression. Genes selected for validation are involved in BCR signalling (*CSK, PTEN, CD37*) and endoplasmic reticulum processes (*SSR* genes, *STT3A*). CD20 upregulation and restoration of RTX cytotoxicity was **validated in *CSK*^{KO} and *SSR1*^{KO} single cell clones and in both *CD37*^{KO} polyclonal population** and single cell clones. Additionally, we looked at CD37 expression in CD20^{KO} cells to better understand the connection between these two surface antigens. Surprisingly, **CD37 was decreased not only in CD20^{KO} cells** but in CD20^{low} cells too. Our data suggest that CD20 and CD37 regulated each other and that CD20^{low} cells might not respond to anti-CD37 therapy.

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