Abstract #26 | Poster

Targeting N-linked Glycosylation for the Therapy of Aggressive Lymphomas

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Diffuse large B-cell lymphoma (DLBCL) is the most common non-Hodgkin lymphoma. Gene expression profiling revealed two major biological subtypes, activated B-cell like (ABC) and germinal center B-cell-like (GCB) DLCBL. ABC tumors rely on self-antigen-induced clustering of the B cell receptor (BCR), invoking chronic active signaling to NF-kB and/or PI3 kinase pathways and require Interferon regulatory factor 4 (IRF4) for their survival. IRF4 is a robust indicator of BCR signaling and an NF-kB target gene.

We devised genome-wide CRISPR-Cas9 screens to identify regulators of IRF4. In addition, using functional (glyco)proteomics, and super-resolution microscopy, we discovered a novel, glycosylation-dependent pathway that inhibits oncogenic BCR signaling.

In our IRF4-GFP CRISPR screens, knockout of BCR pathway components decreased IRF4 expression. Conversely, IRF4 was upregulated by inactivation of negative regulators of BCR/NF-kB signaling. Unexpectedly, IRF4 expression was significantly diminished by inactivation of the non-panessential N-linked oligosaccharyltransferase (OST) B complex, a multiprotein complex transferring a core glycan to asparagine residues of membrane and secreted proteins (Fig. 1).

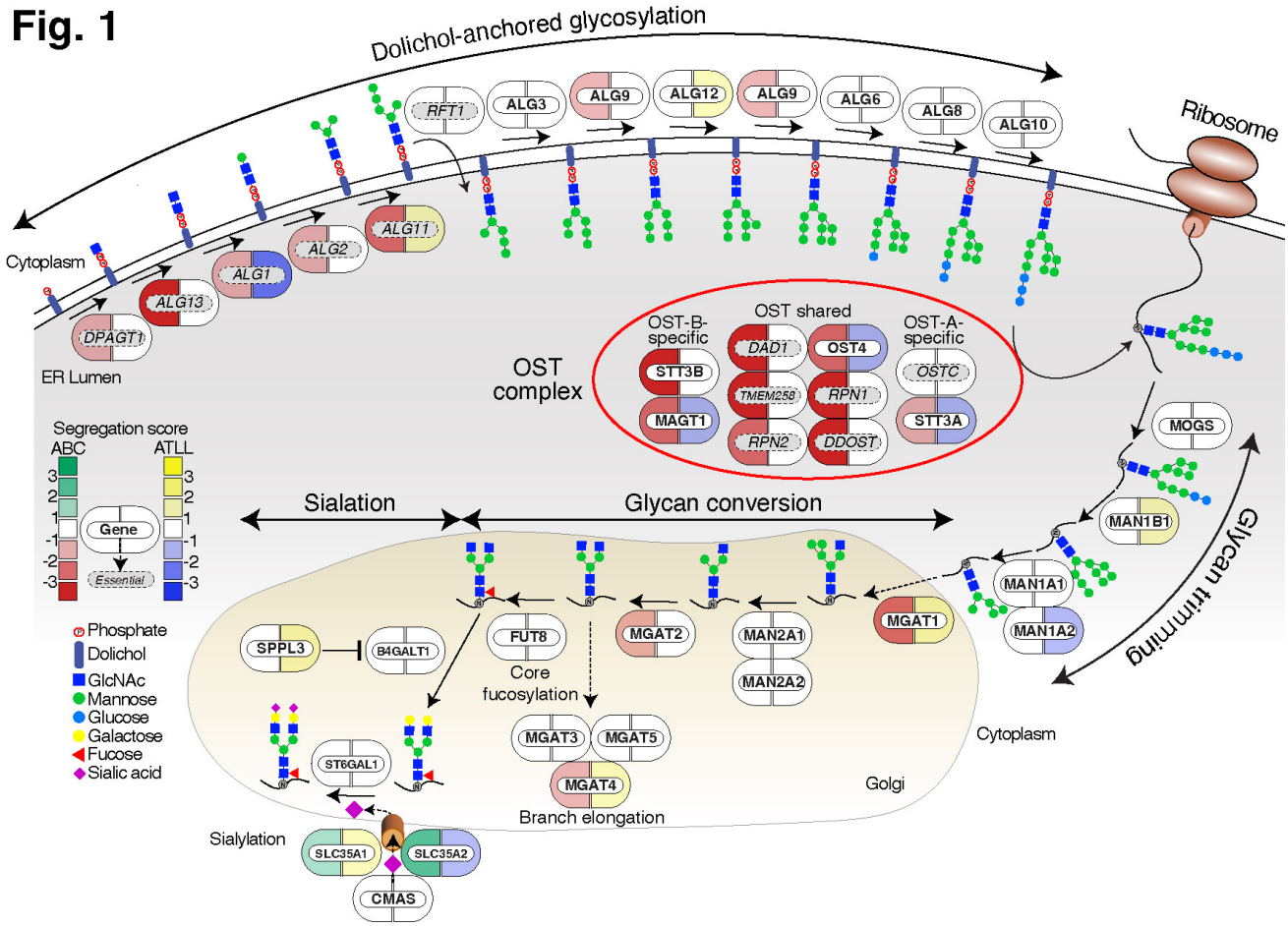
To determine whether lower IRF4 levels after OST-B loss occurred due to regulation of IRF4 protein or via interference with NF-kB signaling, we performed RNA-sequencing utilizing the OST inhibitor NGI-1. IRF4 mRNA was downregulated after NGI-1 supporting the notion that OST inhibition impairs BCR signaling.

To investigate the mechanisms responsible for OST-dependency, we identified proteins that are glycosylated by OST-B and are also critical for lymphoma cell viability. We observed that the essential BCR subunits CD79A and CD79B were strongly altered in its glycosylation. To study the fate of the deglycosylated BCR, we implemented dSTORM microscopy and found a remarkably altered BCR cell surface organization revealing lower clustering of the BCR after NGI-1 treatment.

To study consequences of the altered BCR distribution, we performed mass spectrometry based

interactome analysis of the BCR. The most striking changes in the BCR interactome occurred on the inhibitory co-receptor CD22 being recruited to the BCR after NGI-1 treatment.

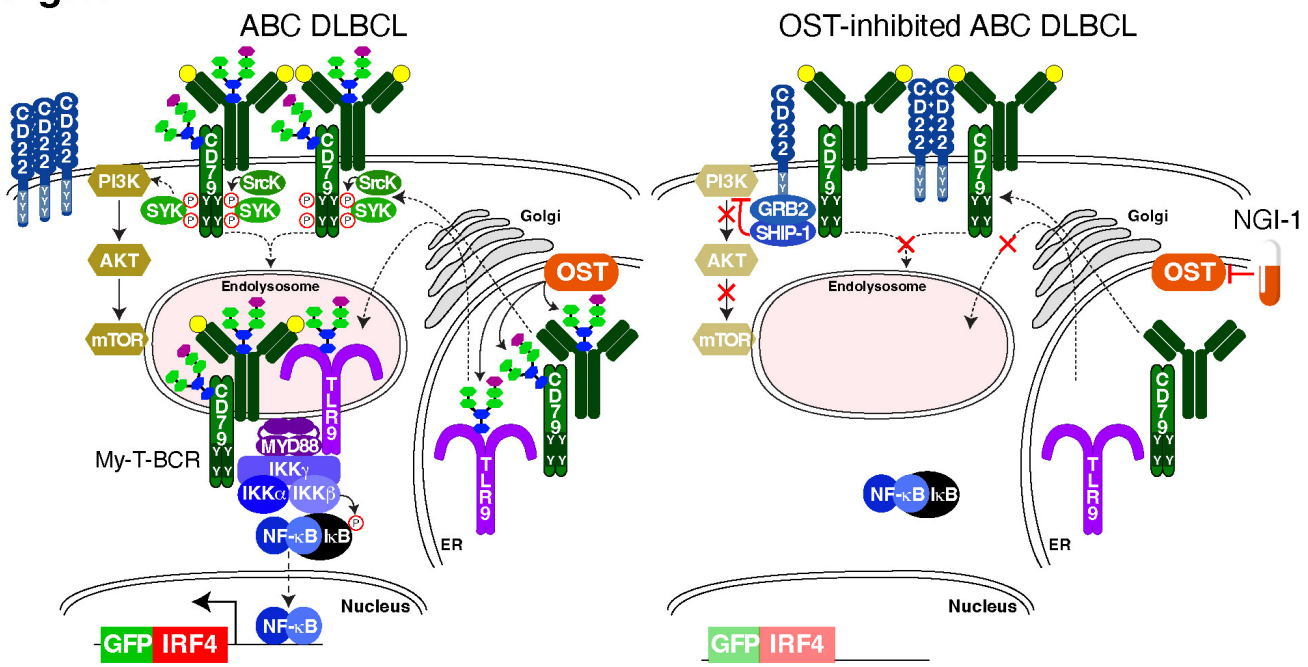
Altogether, our investigations have uncovered a novel pathway regulating oncogenic BCR signaling (Fig. 2) supporting the development of selective OST-B inhibitors in lymphoma.



Glycosylation pathway

Diagram of the N-linked glycosylation, fucosylation, and sialylation pathway. Icons indicate effects on IRF4 protein levels assessed by IRF4–GFP CRISPR screens in ABC cells (left, red to green) or ATLL cells (right,blue to yellow). Pan-essential genes (by DepMap) are marked in italic/gray.

Fig. 2



OST model

Model of OST regulation of oncogenic signaling in lymphoma. PI3K, PI3 kinase.