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ORAL ABSTRACTS

603.LYMPHOID ONCOGENESIS: BASIC

Dynamic Recruitment of Inhibitory Complexes Controls Oncogenic Signaling in B-Cell Malignancies

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Background and Significance: Initiation of B-cell receptor (BCR) signaling, and subsequent antigen-encounter in germinal centers represent milestones of B-lymphocyte development that are both marked by sharp increases of CD25 surface-expression. Oncogenic signaling in B-cell leukemia (B-ALL) and lymphoma also induced CD25-surface expression. While CD25 is known as an IL2-receptor chain on T- and NK-cells, the significance of its expression on B-cells was unclear. High expression levels on the surface of B-ALL cells are associated with poor clinical outcomes in pediatric (COG P9906) and adult (ECOG E2993) B-ALL cohorts. In DLBCL, aggressive disease is associated with CD25-inactivation by shedding and high serum levels of soluble CD25.

Results: Our experiments based on genetic mouse models and engineered patient-derived xenografts revealed that, rather than functioning as an IL2-receptor chain, CD25 expressed on B-cells assembled an inhibitory complex including PKCδ, immunoreceptor tyrosine-based inhibitory motif (ITIM)-bearing receptors and inhibitory phosphatases for feedback control of BCR-signaling or its oncogenic mimics. Recapitulating phenotypes of genetic ablation of PKCδ and phosphatases, conditional CD25-deletion decimated early B-cell subsets but expanded mature B-cell populations and induced autoimmunity. In B-cell malignancies arising from early stages of B-cell development (e.g. B-ALL) CD25-loss induced hyperactivation of oncogenic signaling followed by cell death. In contrast, genetic deletion of CD25 in models for mature B-cell lymphoma accelerated proliferation of malignant cells.

Biochemical and interactome studies revealed a critical role of CD25 in feedback regulation of oncogenic signaling in B-cell malignancies: Signaling from BCR-ABL1 and RAS-oncogenes in B-ALL as well as oncogenic BCR-signaling in DLBCL induced PKCδ-mediated phosphorylation of CD25 on its cytoplasmic tail (S ^{268}). Importantly, CD25-phosphorylation stabilized its expression at the cell membrane and opposed both inactivation by CD25-shedding and internalization. Moreover CD25-S ^{268} tail-phosphorylation enabled CD25 to recruit inhibitory receptors, including CD22, that bear ITIM-motifs for the activation of inhibitory phosphatases (e.g. SHP1, **Figure 1**). Thereby, the positively charged cytoplasmic tail of CD25 engages negatively charged residues in the cytoplasmic tails of the BCR signaling chain CD79B and the inhibitory CD22-ITIM. Molecular dynamics simulations revealed that the ternary complex between CD25, CD79B and CD22 enables recruitment and activation of the phosphatase SHP1 within reach of its substrate, the BCR signaling chain CD79B. Thereby, the SH2-domain of SHP1 engages the CD22-ITIM motif, while its phosphatase domain makes contact with the CD79B-ITAM to dephosphorylate and terminate BCR-signaling. This complex, while engaging and activating SHP1, is held together by CD25 (**Figure 1**).

To test functional consequences of destabilization of this complex, we generated a knockin mouse model carrying a single point mutation (S268A) in the cytoplasmic tail of CD25. The CD25 ^{S268A} knockin mutation abolished recruitment and activation of SHP1. In addition, the S268A-mutant failed to assemble CD22 and CD79B in complex with SHP1 to limit duration and strength of BCR-signaling. Loss of phosphatase-function and failure to terminate oncogenic signaling resulted in constitutive activation of ERK and NF-κB, autonomous Ca $^{2+}$-oscillations. Hyperactivation of oncogenic signaling resulted in exhaustion and cell death in B-ALL cells, as opposed to excessive proliferation and acceleration of disease in mature B-cell lymphoma.

Conclusions: While CD25 has an established function in IL2 signal transduction in T- and NK-cells, these findings highlight the previously unrecognized role of CD25 in assembling inhibitory phosphatases to control oncogenic signaling in B-cell

malignancies and provides an explanation for the common feature of CD25 shedding and high serum levels of functionally inactive soluble CD25 in aggressive B-cell lymphomas.

Disclosures No relevant conflicts of interest to declare.

Figure 1: CD25 assembles an inhibitory complex for feedback control of oncogenic BCR-signaling in B-cell lymphoma

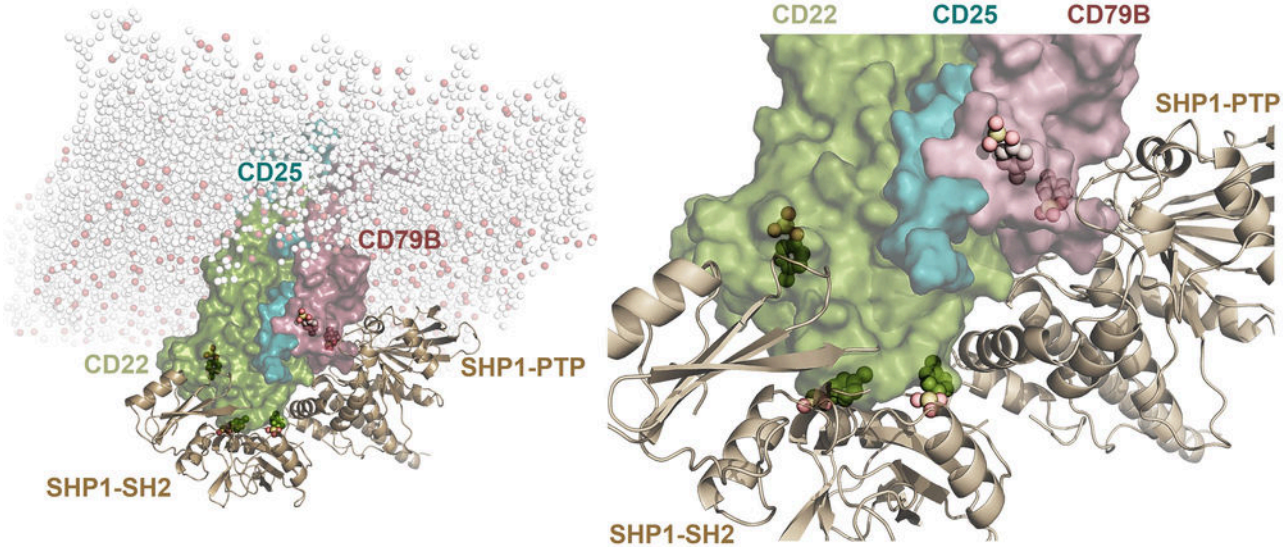

Figure 1

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