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Blood 142 (2023) 4141

The 65th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

603.LYMPHOID ONCOGENESIS: BASIC

Role of Autoantigen Induced-B-Cell Receptor Internalization and Its Inhibition for the Biology and Survival of Diffuse Large B-Cell Lymphoma (DLBCL) Cells

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In normal B-cells, antigen binding by the B cell receptor (BCR) triggers rapid BCR clustering, initialization of signaling, and subsequent endocytosis of the BCR-antigen complex. Numerous studies showed that BCR signaling is continued as the BCR is trafficked intracellularly. Similarly, the internalized BCR in complex with TLR9 in DLBCL cells elicits crucial pro-survival signaling for DLBCL cells. Despite these observations, the role of endocytosis in generating a pathological signal in DLBCL, and a potential of endocytosis blockade as a strategy to inhibit BCR signaling, have not been defined.

To address these questions, we generated DLBCL cell models with modified, ovoalbumin (OVA)-specific hypervariable regions (HVR) in BCR using the knock-in CRISPR-Cas9 methodology. This model allows to study BCR receptor internalization and the signaling that accompanies this process in highly controlled conditions. The change of BCR specificity led to inhibition of spontaneous (autoantigen-initiated) BCR internalization, observed as an increased surface BCR level, and lower proliferative potential of autoautigen-dependent U2932 and HBL1 cells, but not in cells in which autoantigens were not detected (LY19). Using the kinome analysis platform (PamGene technology), we identified kinases activated by BCR-autoantigen binding, including proximal mediators of BCR signaling, cyclin-dependent kinases, protein kinase C and S6 ribosomal kinases.

Simultaneous analysis of the OVA-induced BCR internalization kinetics and signaling activation in the above-described models indicated that the activation of certain signaling cascades may emanate from the inside of the cell. Using the Proximity Ligation Assay (PLA) method, we identified the presence of BCR-TLR9-IkB complexes (activators of the NF-kB transcription factor), in the endosomal compartment of autoantigen-dependent DLBCL cells. Cells with OVA-specific BCR showed a drastically decreased number of BCR-TLR9-IkB complexes, which was associated with lower expression of NF-kB-dependent genes.

To confirm the crucial role of the autoantigen-induced BCR endocytosis in sustaining the signaling and promoting DLBCL cells survival, we reanalyzed available data from the CRISPR-Cas9-Screen studies. This approach revealed the key role of genes involved in endocytosis, including dynamin-2, for DLBCL cell survival. Based on these observations, we generated cell models characterized by inducible expression of the dominant-negative (DN) form of dynamin-2, which inhibits dynamin-2-dependent endocytosis. Experiments performed using these models showed that BCR internalization is dependent on dynamin-2, and the induction of the DN-dynamin-2 form blocks internalization of the BCR receptor, BCR-TLR9-IkB complexes assembly and activation of BCR-initiated signaling pathways, including the transcription factor NF-kB, which markedly inhibited the growth of DLBCL cells. In addition, clinically available Dynamin-2 inhibitors (e.g. certain phenothiazine derivatives), also inhibited BCR endocytosis and were toxic to DLBCL cells.

In summary, our observations demonstrate that autoantigen-induced endocytosis of the BCR receptor is the key mechanism supporting the BCR signal and ensuring the survival of DLBCL cells with autoantigen-specific BCRs. Furthermore, we show that endocytosis is a rational therapeutic target in this group of lymphoid malignancies.

Disclosures No relevant conflicts of interest to declare.

https://doi.org/10.1182/blood-2023-187046