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Title: ANTIGEN-INDEPENDENT, CELL-AUTONOMOUS SIGNALING IN SPLENIC MARGINAL ZONE LYMPHOMA

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Background:

The B cell receptor (BcR) is retained in most malignancies of mature B cells, hence implicating triggering by antigen(s) in their natural history. Chronic lymphocytic leukemia (CLL) is a prototype for the role of antigen selection as a driver of disease pathogenesis. This notion was recently questioned by the demonstration that antigen-independent, cell-autonomous signaling, a form of tonic signal generated through the self association between BcR expressed on adjacent leukemic cells, may represent a crucial pathogenic mechanism. Similar to CLL, antigen selection is relevant also in splenic marginal zone lymphoma (SMZL), another mature B cell malignancy. However, in contrast to CLL, cell-autonomous signaling was not detected previously in SMZL. That said, the existing evidence was based on only 3 cases precluding definitive conclusions from being drawn.

Aims:

Here, we explored the existence of antigen-independent cell-autonomous signaling in SMZL.

Methods:

The clonotypic IG heavy and light chain cDNAs of 17 SMZL cases were cloned into retroviral vector as a single ORF joined by cleavable P2A peptide, which was then expressed in a ERT2-SLP65 expressing, RAG2, λ5 and SLP65 triple knockout (TKO) mouse cell line. BcR expression was verified by FACS and autonomous signaling analyzed by Calcium (Ca^{2+}) influx upon stabilizing the ERT2-SLP65 by 2μM 4-Hydroxytamoxifen (4-OHT) stimulation of cells preloaded with Indo-1 AM dye, a high affinity, intracellular Ca^{2+} indicator reporting ratiometric changes in the fluorescent intensities at variable cytoplasmic Ca^{2+} concentration. To confirm BcR signaling competence, cells were additionally treated with anti-IG antibodies resulting in BcR cross-linking. We categorized the outcome of autonomous BcR signaling in three qualitative classes, namely absent (no autonomous BcR signaling in all or >2 independent transductions), intermediate (weak and repeatable autonomous BcR signalling in >2 independent transductions) and, finally, strong (robust and repeatable autonomous BcR signaling in independent measurements). Moreover, we performed statistical analysis by calculating the mean difference of the area under the curve (AUC) values between the SMZL BcR cases and the negative control (healthy PBMCs) derived from several individual replicates.

Results:

We investigated 17 cases expressing BcR IG encoded by the IGHV1-2*04 (n=5) or other IGHV genes (n=12); of note, the former accounts for ~30% of all SMZL cases. The studied cases displayed variable imprints of somatic hypermutation (SHM) in the IGHV genes, ranging from absent (n=6) to minimal/low (n=6) to extensive (n=5), reflecting the distribution observed in the SMZL repertoire. Of the 17 tested SMZL BcR, 8 showed strong (mean difference of the AUC value ranged from 36,109 to 82,045) and 3 displayed weak BcR autonomous signal upon activation of SLP65 by 4-OHT (mean difference AUC ranged from -2,212 to 29,871); the remaining 6 cases did not show autonomous signaling (mean difference of the AUC ranged from -5,734 to 10,018). All tested SMZL BcR were responsive to BcR cross-linking (anti-Igκ/λ depending on the isotype expressed or anti-IgM). The pattern of autonomous signaling observed in SMZL showed no correlation with IGHV gene usage and/or IGHV gene SHM status.

Summary/Conclusion:

We demonstrate for the first time that a large fraction of SMZL BcR engage in cell-autonomous signaling, independent of the presence of specific extrinsic antigens. This observation supports a potentially crucial pathogenetic mechanism implicating intermolecular BcR-BcR interactions in the pathogenesis of SMZL.

Keywords: Splenic marginal zone lymphoma, Signaling