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ORAL ABSTRACTS

603.LYMPHOID ONCOGENESIS: BASIC

Mechanistic Elucidation of the Tumor-Promoting Role of Carcinoembryonic Antigen-Related Cell Adhesion Molecule 1 in B-Cell Receptor Signaling in Mantle Cell Lymphoma

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Mantle cell lymphoma (MCL) is a highly aggressive and incurable type of B-cell non-Hodgkin's lymphoma. B cell receptor (BCR) signaling plays an important role in the pathogenesis of MCL; however, the underlying mechanisms are unknown. Our genome-wide screens identified that carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1/CC1) is a central component of BCR signaling and is essential for MCL survival in vitro and in vivo. Among the twelve alternatively spliced human CC1 isoforms, we found that CC1-4L, the longest CC1 isoform is overexpressed in MCL. Structurally, it has the N-terminal domain and three type 2 constant ectodomains, a transmembrane sequence and a signaling cytoplasmic domain with two immunoreceptor tyrosine-based inhibitory motifs (ITIMs or Y493/520). These ITIMs of CC1 can inhibit signals in T cells, NK cells, neutrophils and granulocytes. However, its role in B cells and BCR signaling is not clear.

In this study, we have elucidated a novel mechanistic function of CC1 in promoting oncogenic BCR signaling in MCL. First, we studied the role of CC1 in lipid raft formation, which is one of the early events during BCR signaling activation. To study this, we used three panels of MCL lines, Jeko-1-NTC (non-template control), Jeko-1-gCC1 (CC1-knockout) and Jeko-1-gCC1-4L (CC1-knockout restored with CC1-4L isoform). Using high-resolution confocal microscopy, we observed that CC1-expressing Jeko-1 MCL cell line showed significantly increased lipid raft formation when compared to CC1-knockout cells. However, CC1-deficient cells restored with the CC1-4L isoform exhibited increased lipid raft assembly. Further, we also observed that CC1-expressing MCL lines showed increased cytoskeletal assembly, which was observed via increased F-actin and filamin A staining in CC1-expressing MCL lines when compared to CC1-knockout cells.

Second, we analyzed the phosphorylation levels of proteins involved in the BCR signaling cascade in a panel of genetically engineered MCL lines, i.e., Jeko-1-NTC, Jeko-1-gCC1, Jeko-1-gCC1-4L, Jeko-1-gCC1-4S (CC1-knockout restored with CC1-4S or short cytoplasmic tail isoform) and Jeko-1-gCC1-mutants (CC1-knockout cells restored with N-terminal domain deletion mutant and ITIM-mutant or YY/FF $^{493/520}$). We observed that Jeko-1-NTC and Jeko-1-gCC1-4L cell lines showed significantly increased phosphorylation levels of BCR signaling components such as phospho-SRC (Y416), phospho-SYK (Y319), phospho-AKT (S473) and phospho-BTK (Y223) when compared to Jeko-1-gCC1, Jeko-1-gCC1-4S and Jeko-1-gCC1 mutant MCL lines. This shows that CC1 positively regulates or activates oncogenic BCR signaling in MCL and the N-terminal domain and cytoplasmic tail are required for this function.

Next, we analyzed whether CC1 could interact with the BCR signaling components such as CD79B, LYN and SYK using immunoprecipitation (IP) and proximity ligation assay (PLA) in MCL lines. Further, we analyzed the kinetics of BCR activation in order to infer the role of SHP-1 binding to the ITIMs of CC1. Our IP and PLA experiments demonstrated that in Jeko-1 and Mino MCL lines, at the peak (i.e., at 5 and 15 min) of BCR activation, there was significantly increased interaction between CC1 and the BCR-pathway components (CD79B, LYN and SYK), and this interaction subsided by 30 min of IgM activation. However, in the case of CC1 and SHP-1, the interaction was stable throughout the time points and increased by 30 min of IgM activation. These interactions were disrupted in the CC1-N-terminal domain deletion and ITIM mutant MCL lines when compared to the CC-4L expressing MCL line. Finally, we analyzed the interaction between CD79B and SYK in CC1-4L, CC1-ITIM mutant and N-domain deletion mutant expressing MCL lines. Using PLA, we observed increased interaction between

CD79B and SYK in CC1-4L expressing cell line compared to other cell lines indicating that functional CC1 is required for the recruitment of SYK to CD79B.
In summary, CC1 stabilizes lipid rafts following BCR engagement by anchoring to F-actin and Filamin A. CC1 recruits and activates LYN and SYK at the BCR complex at the peak of BCR activation and subsequently recruits SHP-1 for signal downregulation. These activities of CC1 require intact ITIMs and the N-terminal ligand-binding domain. Hence, CC1 drives oncogenic BCR signaling in MCL and forms an important candidate for MCL immunotherapy.

Disclosures No relevant conflicts of interest to declare.

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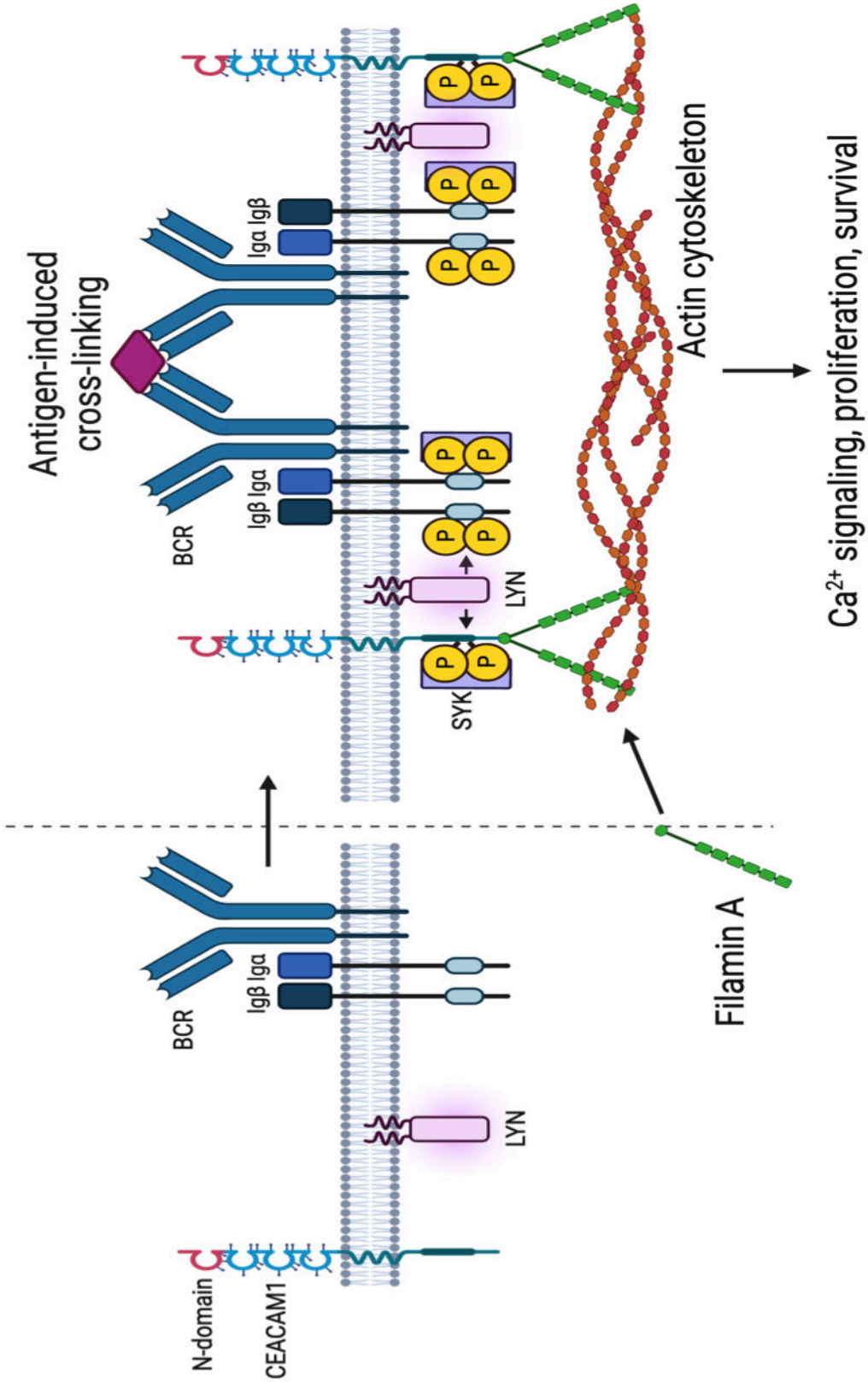

Figure 1