The Potential Role of miR-29c-3p in Regulating TNF Signaling in Chronic Lymphocytic Leukemia

Chronic lymphocytic leukemia (CLL) is a lymphoproliferative disorder characterized by progressive accumulation of monoclonal CD5+ B cells. CLL is classified into two subgroups based on the somatic hypermutation (SHM) status of the immunoglobulin heavy variable (IGHV) gene. These subgroups, IGHV unmutated (U-CLL; ≥98% germline homology) and IGHV mutated (M-CLL; <98% germline homology), are biologically and clinically different. As genetic and gene expression analyses have not fully captured these differences, we turned to microRNA analyses.

We conducted microRNA sequencing on CD19+ B cells from 23 treatment-naïve CLL patients (M-CLL, n = 15; U-CLL, n = 8) using samples from a multicenter biobank in the Netherlands. MicroRNA sequencing revealed significantly higher expression of miR-29c-3p in M-CLL (log2FC = 0.97, P = 0.005), which was validated using qRT-PCR on a different set of samples (M-CLL, n = 22; U-CLL, n = 9; log2FC = 1.46 [95% CI = 0.373 – 2.55], P = 0.013). In silico analysis suggested that miR-29c-3p regulates tumor necrosis factor (TNF)-related pathways by suppressing TNFRSFR1A, which encodes TNF receptor 1 (TNFR1). TNFR1 and TNFR2, the latter encoded by TNFRSF1B, both are receptors for TNF, but they trigger different biological responses. TNFR1 can induce apoptosis, whereas TNFR2 is more prone to activate survival pathways. To support our in silico predictions, we conducted qRT-PCR experiments on additional samples (M-CLL, n = 7; U-CLL, n = 7), which confirmed that TNFRSF1A is significantly downregulated in M-CLL compared to U-CLL (log2FC = -1.76 [95% CI = -3.108 – -0.405], P = 0.017). In contrast, TNFRSF1B levels showed no significant difference (log2FC = -0.31 [95% CI = -1.504 – 0.893], P = 0.577). As a result, the TNFRSF1B to TNFRSF1A ratio was higher in M-CLL (1:0.51) than in U-CLL (1:1.10, P = 0.032). These findings were corroborated by a large external transcriptomic dataset (n = 471). Our current work focuses on investigating the mechanistic role of miR-29c-3p in TNFR1 signaling using functional assays.

Our data suggest that miR-29c-3p is a regulator of TNFR1 signaling in M-CLL cells. We hypothesize that the response of CLL cells to TNF varies based on their IGHV SHM status. In this tentative model, M-CLL cells are TNFR2-dependent, and this is achieved by miR-29c-3p-mediated inhibition of TNFR1-induced pro-apoptotic signaling. Contrastingly, U-CLL cells may not require such a mechanism, as their main proliferative signal is received in an antigen-dependent manner, predominantly via B cell receptor and Toll-Like Receptor stimulation. This may explain differences in clinical course of patients with M-CLL and U-CLL.

Overall, our findings highlight miR-29c-3p as a novel regulator of TNFR1 in M-CLL, with potential implications for understanding CLL pathogenesis and developing targeted therapies.