Aberrant expression of Oct-2, Spi-B, and Id2 is associated with repression of plasma cell differentiation in Waldenström's Macroglobulinemia.

Yangsheng Zhou, Xia Liu, Lian Xu, Guang Yang, Yang Cao, Zachary R. Hunter, Christopher Patterson, and Steven P. Treon.

Bing Center for Waldenström's Macroglobulinemia, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA, USA.

Waldenström's macroglobulinemia (WM) is a lymphoplasmacytic lymphoma characterized primarily by tumor infiltration of lymphoplasmacytic cells (LPC) in the bone marrow (BM) and presence of an IgM monoclonal gammopathy. WM LPC exhibit deficiency in the ability to fully differentiate to plasma cells. We therefore analyzed the expression of several genes involved in B-cell differentiation by real time RT-PCR, including Ets factors, basic helix-loop-helix (bHLH) E proteins, as well as inhibitors of DNA binding (Id) proteins which antagonize E protein activity. Comparison of bone marrow $CD19^+$ B cells obtained from 12 untreated WM patients versus 15 age-matched healthy donors showed that in WM LPC, expression of the Ets factor Spi-B, Id2, and Id1 was increased four-fold, decreased three-fold and ten-fold, respectively, while transcript levels of E proteins were similar between these two groups. Following cytokine induced differentiation of primary $CD19^+$ cells from healthy donor peripheral blood into $CD38^+CD20^-$ plasmablasts, we observed that Spi-B and Id2/Id1 expression levels were significantly decreased and increased, respectively. Furthermore, ectopic expression of Spi-B in primary $CD19^+$ inhibited plasma cell differentiation which was associated with decreased transcription levels of BLIMP1, XBP-1 spliced form, and IRF4. Over-expression of Spi-B in BCWM.1 WM cells also resulted in repressed expression of BLIMP1, XBP-1 spliced form, and IRF4. Conversely, knocking down of Spi-B in BCWM.1 WM cells increased IRF4, Id2, and Id1 expression. Importantly, in lentiviral transduced primary WM bone marrow $CD19^+$ cells, knocking down of Spi-B induced $CD38^+CD20^-$ plasmablast formation which was related to increased expression of BLIMP1, XBP-1 spliced form, IRF4, and Id2. Moreover, knocking down of Spi-B in primary WM LPC led to decreased Bcl-2 expression. Since in mice Spi-B is a direct target of OBF-1, which forms a ternary complex with the POU proteins Oct-2 or Oct-1 to interact with the conserved octamer site in promoter region, we next evaluated their roles in WM. While transcript levels of OBF-1 and Oct-1 were similar, transcript levels of Oct-2 were three-fold higher in WM LPC versus healthy donors. Knocking down of Oct-2 in BCWM.1 WM cells decreased Spi-B, Id2, and Id1 expression. In addition, chromatin immunoprecipitation (ChIP) confirmed the presence of Oct-2 and OBF-1 in the human *Spi-B* and *Id2* promoter region. These data suggest that Oct-2 and OBF-1, in concert with Spi-B, regulate the transcription of Id2 during B-cell differentiation. These findings establish for the first time the molecular hierarchy among Oct-2, Spi-B, and Id2 in human B-cells. The results also suggest that aberrant expression of these transcription factors plays a critical role in the pathogenesis of WM by repressing factors involved in plasma cell differentiation while promoting WM LPC survival through Bcl-2.