Development of Waldenstrom Macroglobulinemia Cell Lines

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Waldenstrom's macroglobulinemia (WM) is a rare B-cell lymphoproliferative disorder characterized by unique morphological and functional properties. In this disease, there is typically an infiltrate in the bone marrow of lymphoplasmacytic cells consisting of small lymphocytes and plasma cells. The malignant cells are typically indolent with a very slow proliferative rate and they secrete high levels of monoclonal IgM protein. Understanding the molecular mechanisms that contribute to WM pathogenesis has been challenging due to the fact that WM is a rare disease and acquisition of patient derived tumor tissue for research is infrequent. Additionally, development of a WM cell line that models the disease for research studies has proven extremely challenging primarily due to the low proliferative rate of the tumor cells and the fact that primary WM cells do not naturally grow in culture. There are currently 3 cells lines that have been reported to model WM, however none of the cell lines have proven to be both clonally related to the patients tumor by IgH-sequence analysis and secrete IgM. Therefore, there is currently a great need for development of WM cell lines to further our understanding of this disease. The objective of this study was to develop and characterize WM cell lines that are biologically stable and immortalized, but functionally retain characteristics of the original tumor. To generate cell lines, bone marrow samples from WM patients were lysed with Ack lysis buffer to remove erythrocytes and a subset the cells were immediately frozen for RNA isolation. The remaining cells were either put directly in culture or sorted for CD19+ and CD138+ cells and then put in culture. Cells were grown in Iscove Modified Dulbecco Medium GlutaMAX supplemented with 50 U/mL penicillin G, 10 µg/mL gentamicin, 50 µg/mL streptomycin, 10% heat-inactivated fetal calf serum, 1 ng/ml IL-6, 10 ng/ml IGF, and 50 ng/ml BAFF. Cells were monitored daily and media was replaced as needed. To date, bone marrow biopsies from 7 patients have been cultured in this manner and 3 have expanded in number for further analysis. Biologic and genetic characterization of each potential cell line consisted of; EBV screening by EBNA PCR, surface receptor profiling by flow cytometry (IgL kappa and lambda, CD19, CD20, CD3, CD27, CD38, CD138, BCMA, and TACI), IgM secretion measured by ELISA, and IgH CDR3 analysis by PCR and sequencing. WCL1 was derived from unsorted tumor cells, it expresses CD19, CD20, CD27, CD38, CD138, BCMA, and TACI, it secretes IgM, and is EBV positive. IgH CDR3 analysis of WCL1 and the original tumor cells reveal

that both are VH3-15 with identical CDR3 sequences and both express kappa light light chain suggesting that they are clonally related. WCL2 was derived from unsorted tumor cells, it expresses CD19, CD20, CD27, CD38, BCMA, and TACI, it secretes low levels of IgM, and is EBV positive. IgH CDR3 analysis is in progress, however both WCL2 and the original tumor cells express lambda light chain. WCL3 was derived from CD19+CD138+ tumor cells, it expresses CD19, CD20, CD27, CD38, CD138, BCMA, and TACI, it secretes IgM, and is EBV negative. IgH CDR3 analysis of WCL3 and the original tumor cells reveal that both are polyclonal with some identical sequences and both express kappa light chain. Further CDR3 analysis of this sample is ongoing. To date, development of a WM cell line has proven to be difficult. Our experience has been similar, and our initial data suggest that WCL2 and WCL3 most likely represent an outgrowth of normal B cells that are not related to the WM clone. However, WCL1, although EBV positive, may represent a useful model for study of WM and experiments to further characterize this potential cell line are ongoing.