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POSTER ABSTRACTS

621.LYMPHOMAS: TRANSLATIONAL-MOLECULAR AND GENETIC

Development of Syngeneic Murine Cell Lines of Germinal Center-Derived B-Cell Lymphomas

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Background. Germinal center (GC)-derived B-cell lymphomas are clinically and biologically heterogeneous. Next generation sequencing has enabled identification of genetic aberrations involved in lymphoma pathogenesis, which may provide prognostic predictions and potential therapeutic targets. However, the mechanisms driving pathogenesis are still not fully understood, especially in the context of an intact tumor microenvironment, in part because there are no available syngeneic models of lymphoma faithfully representing the pathological features of human disease. Therefore, there is a critical need for lymphoma models which can be studied in immunocompetent mice.

Methods. We established eight murine cell lines derived from spontaneous B-cell lymphomas with GC origin arising in genetically engineered mouse models (GEMM). These cell lines were developed by passing an original tumor from a GEMM through several passages of Rag1-/- and wild type mice, which enabled selection of more fit/aggressive clones that grow in vitro. We also tested multiple culture conditions and characterized the lines via flow cytometry, VDJ and targeted sequencing, karyotyping, and evaluation of tumor development and its microenvironment *in vivo*.

Results. This resource describes eight cell lines driven by recurrent genetic mutations found in diffuse large B-cell lymphoma and follicular lymphoma patients. The first cell line is defined by (1) BCL2 overexpression (hereafter B2). Four cell lines couple BCL2 overexpression with epigenetic dysregulation, defined by (2) BCL2 and gain-of-function (GOF) mutation of EZH2 ^{Y641F} (hereafter EZB), (3) EZB and MYC overexpression (hereafter EBM), (4) BCL2 with knockout of KMT2D (hereafter KB), and (5) BCL2 with knockout of SETD2 (hereafter S2B). Finally, three cell lines are defined by mutations to the Toll-like and B-cell receptors pathways, defined by (6) BCL2 and GOF mutations in Myd88 ^{L252P} and CD79b ^{Y195H} (hereafter MBC79), (7) BCL2, Myd88 ^{L252P} and GOF mutation in Card11 ^{L251P} (hereafter MBC11), and (8) BCL2 and Card11 ^{L251P} (hereafter BC11).

Doubling time of these lines ranges from 30-72 hours, with EZB and EBM on the fast end and S2B on the slow end. By flow cytometry, we show that B2, MBC79, and MBC11 lose B-cell surface markers and are very plasmacytic (>95% B220- CD19- CD138+). In contrast, the EZB, EBM, KB, BC11, and S2B are mostly characterized by GC B-cell markers (B220+ CD19+ CD138- FAS+ CD38- GL7+). By VDJ sequencing, we found that EZB, BC11, and S2B are very monoclonal, with one rearrangement arising from the original tumor and taking over to represent >95% of clones. In contrast, the MCB11 and MBC79 lines are very polyclonal, with the top 10 rearrangements being equally represented, and tending to shift between passages.

Thus far, S2B and EZB have been shown to grow in immunocompetent mice, representing novel syngeneic lymphoma models. While mice injected with S2B can survive for up to 30 days, mice injected with EZB die significantly earlier. We established two EZB lines from early (P1) and late (P6) passages of a tumor arising from the same original mouse. Notably, we show that mice injected with P1 survive 15-20 days, whereas mice injected with P6 survive 10-15 days.

Although both P1 and P6 grow at similar rates *in vitro*, P1 develops high grade follicular lymphomas in C57BL6 recipients, whereas P6 develops DLBCL, suggesting additional passaging drives selection for more aggressive disease. Imaging of the tumors reveals a lack of follicular pattern. Notably, P6 tumors display less infiltration of CD4 and CD8 T-cells and low abundance or absence of FDC meshwork, as compared to P1. Imaging of mice engrafted with luciferase transduced P1 and P6 shows dissemination into spleen, lymph nodes, liver, and kidney, with more advanced disease in P6 injected mice.

Conclusion. While conventional preclinical tumor mouse models are often used for drug sensitivity and treatment prediction studies, they are time-consuming and expensive. These cell lines serve as a preclinical tool to test novel therapeutic strategies and develop a deeper understanding of the molecular mechanisms driving lymphomagenesis, therapeutic response, and resistance in genetically defined subtypes of GC-derived B-cell lymphomas. Most importantly, this resource meets a critical need in the field for syngeneic models of lymphoma to study disease progression and precision therapy in immunocompetent mice.

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