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POSTER ABSTRACTS

603.LYMPHOID ONCOGENESIS: BASIC

MYC to BCL6 State-Transitions Determine Cell Size and Metabolic Fluctuations and Define a Novel Biorhythm in B-Cell Malignancies

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Background: Cell size fluctuations are well-documented during critical transitions during normal B-cell development. For instance, during early stages of development, "large cycling pre-B cells" (also classified as Fraction C') eventually exit cell cycle and shrink to become "small resting pre-B cells" (Fraction D) to enable immunoglobulin light chain gene recombination. We and others had shown that this transition is marked by loss of Myc expression and gain of Bcl6. Later in development, germinal center B-cells cycle between large MYC+ "centroblast" and smaller BCL6+ "centrocyte" states between dark and light zones, respectively. MYC is involved in biomass accumulation and provides fuel for cell division, while BCL6 confers a quiescent phenotype to cells and protects B cells from DNA damage-induced apoptosis. BCL6 can suppress MYC transcription, indicating MYC and BCL6 is mutually exclusive, and their expression dictates distinct cellular states.
Significance: In B-cell malignancies, transitions between mutually exclusive MYC+ and BCL6+ states seem to be important as well: B-ALL cells that are driven by oncogenic tyrosine kinases (e.g. BCR-ABL1) express predominantly MYC but can be forced into a BCL6+ state upon tyrosine kinase inhibition (e.g. imatinib). In germinal center-derived B-cell lymphoma, both MYC and BCL6 are frequently targeted by chromosomal translocation, suggesting that disruption of physiological state transitions may be part of the malignant transformation program.
Results: To elucidate regulation of MYC-BCL6 state transitions and their importance in progression and development of drug resistance in B-ALL and germinal center-derived B-cell lymphoma, we developed a MYC-eGFP and BCL6-mCherry dual-reporter mouse model. In addition, we engineered human B-ALL PDX cells with MYC-mNeonGreen and BCL6mScarlet knockin fusion genes by CRISPR/Cas9-mediated HDRT. As expected, MYC+ and BCL6+ states were largely mutually exclusive during early stages of B-cell development, as well as in BCR-ABL1- and NRAS ^{G12D}-driven B-ALL. Interestingly, a large fraction of B-ALL cells expressed neither MYC nor BCL6, as validated by cell sorting and Western blot. Based on single-cell sorting and subsequent time-lapse monitoring over 12 hours, we found MYC+ cells transitioning through a double-negative state to become BCL6+ and then revert again to a MYC+ state (**Figure A**), suggesting dynamic transitions between MYC and BCL6 cycles. We characterized these populations by analyzing cell size, cell proliferation, cell cycle, clone formation, and gene expression profiles. The results indicated that MYC+ cells exhibited larger cell size, active proliferation, and increased glycolytic activity. Conversely, BCL6+ cells displayed smaller cell size, activation of autophagy with suppression of glycolysis, and cell cycle arrest in the G0 phase of the cell cycle.
Conclusions and future directions: Given the dynamic inter-transition between MYC and BCL6 states, and the significant roles of both MYC and BCL6 in B-ALL and germinal center-derived B-cell lymphomas, we propose MYC- and BCL6-dependent fluctuations of cellular activity (wake) and quiescence (sleep; **Figure B**). Thereby, MYC and BCL6 are expressed in an oscillatory manner. During the MYC state, cells exhibit high glycolysis activity, actively accumulate biomass, and undergo proliferation, representing the wake phase. In contrast, during the BCL6 state, cells suppress glycolysis metabolism, pause the cell cycle, and display a quiescent phenotype, representing the sleep phase. Based on this discovery, we will examine the 'B-cell exhaustion' paradigm that Bcl6 and Myc mark iterative cycles of quiescence and activation. In analogy to sleep-wake phases, we hypothesize that the Bcl6-autophagy phase is essential for recovery and regeneration. We will investigate how B-cells transition from one phase to the next and consequences of elongation of one phase at the expense of the other. Malignant B-cells may be particularly sensitive to shortening of quiescence periods ("sleep deprivation"). These and other observations will lead to new conceptual frameworks for the understanding of how MYC-BCL6 state transitions, cell-size fluctuations and the length of recovery-periods regulate energy-supply and survival of B-cell malignancies and create opportunities for therapeutic intervention.

Disclosures No relevant conflicts of interest to declare.

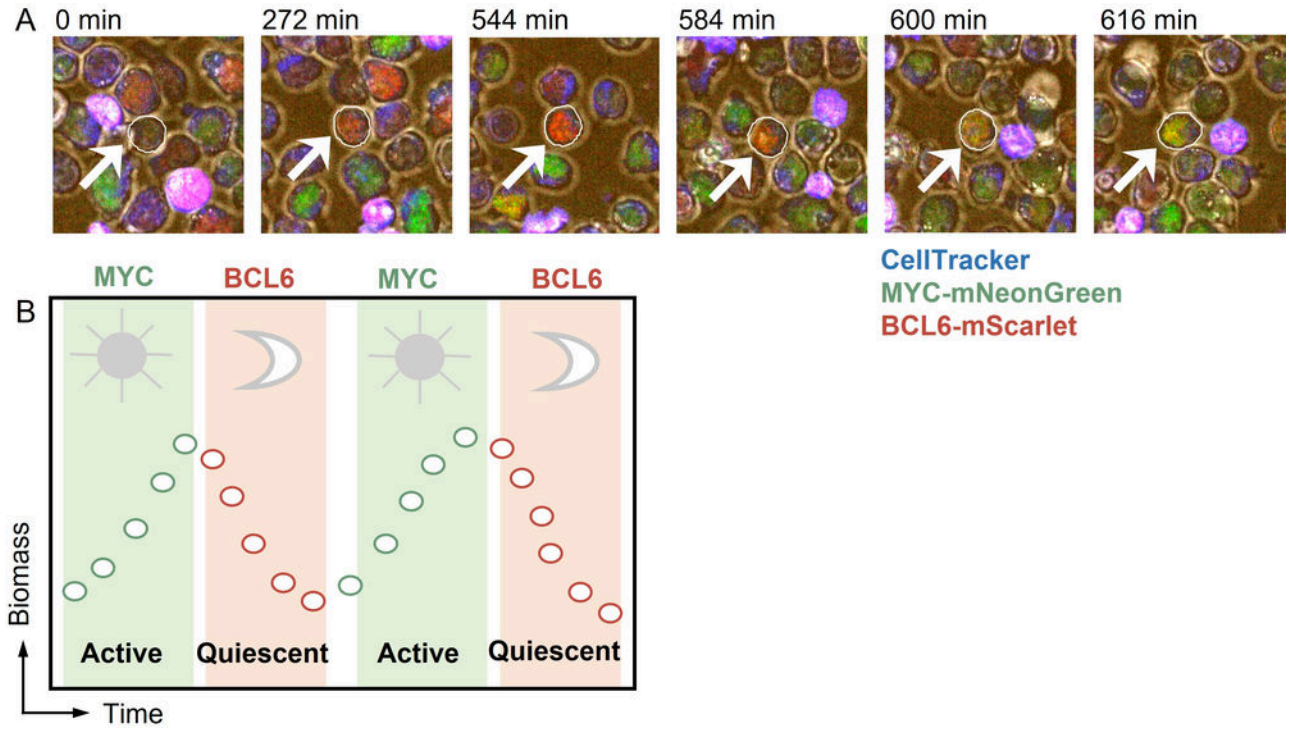

Figure 1

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