Characterization of the Sialic Acid Biosynthesis Pathway in Ba/F3 Cell Transformation

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Ba/F3 is a murine pro-B cell line historically used in mechanistic studies of oncogenic transformation. Ba/F3 cells depend on the cytokine Interleukin-3 (IL-3) for survival and proliferation, without which they undergo apoptosis. A genome-wide pooled-library CRISPR knockout screen was performed in this system to identify novel tumor suppressor genes. Pathway analysis from the hits of this screen identified two genes in the sialic acid biosynthesis pathway: Cmas and Nans. Sialic Acid is a sugar added post-translationally to proteins targeted to cell and organelle membranes. Cell surface sialylated proteins function in signaling, adhesion, migration, immune recognition, and death. Aberrant sialylation is observed in various cancer types. My project aimed to characterize the roles of Cmas and Nans on Ba/F3 cell transformation to further understand the causative relationship between aberrant sialylation and lymphomagenesis. We began by generating Cmas and Nans CRISPR knockouts. Phenotypic characterization by cell surface sialylated protein staining using MAL II, a plant-based lectin which binds specifically to cell surface 2,3-sialic acid linkages, revealed heterogenous expression levels. This necessitated clonal isolation of efficient knockouts. Single cell-derived clones were cultured by limiting dilution, and complete knockouts were identified by western blots. Three clones each were selected for functional analysis by lectin cytochemistry. Following confirmation of homogenous reduction in cell surface sialylation, we analyzed IL-3 dependence. The IL-3 deprivation assay demonstrated enhanced cell survival in both Cmas and Nans knockouts. Further studies are necessary to identify the specific sialylated proteins that contribute to cell survival in the absence of IL-3. Supported by NIH R25ES020721 and R01CA232246.