STRONG CHILDREN'S RESEARCH CENTER

Summer 2019 Research Scholar

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ABSTRACT

Title: Generation of *STAG2* mutations in a murine cell line to study Down syndrome-related myeloid disorders

Background: Transient abnormal myelopoiesis (TAM) is an immature megakaryoblast proliferation that occurs in patients with Trisomy 21. Mutations in *GATA1* lead to the expression of a shorter protein (GATA1s) and are shown to induce a TAM phenotype. Secondary mutations are associated with progression from TAM to Acute Megakaryoblast Leukemia (AMKL). Since *GATA1* is a driver of myeloid differentiation, questions remain about what role additional gene mutations have in the progression of AMKL. While the mechanism of AMKL progression remains unknown, *STAG2* mutations have been associated with the development of this disorder.

Objective: Model systems with *STAG2* mutations are needed to understand the role of *STAG2* in the development of AMKL. Using the CRISPR/Cas9 system, we introduced sgRNAs targeting *STAG2* into Murine Erythroleukemia (MEL) cells. To effectively electroporate the cells and insert the sgRNA, we optimized electroporation conditions. We obtained DNA from the cells and analyzed by Sanger sequencing to determine what genetic changes occurred. Additionally, we analyzed gene expression of *STAG2* in our cell lines, as well as cell imaging flow cytometry to measure morphology and epitope expression in mutated cell lines.

Results: Optimized electroporation conditions successfully introduced mutations in MEL cells at the *STAG2* locus via CRISPR/Cas9, while maintaining cell viability. We found that *STAG2* sgRNA introduced insertions and deletions with 77% efficiency (p-value <0.001). Two lines with verified mutations were selected for further analysis. Mutated cell growth rate was not significantly different compared to the control sample. Gene expression analysis showed a consistent gene knockdown efficiency of 60%-80% for both lines. Interestingly, non-mutated cells didn't seem to overgrow the *STAG2* knockdown, since gene knockdown was consistent two weeks after electroporation. Imaging flow cytometry results showed a subset of cells with increased CD71 surface expression and increased cell size in the mutated cell samples.

Conclusion: Our data shows a successful *STAG2* gene knockdown after the introduction of a sgRNA into MEL cells using optimized electroporation conditions that maintained cell viability. By engineering a known human mutation, linked to a myeloid disorder in patients with Trisomy 21, we have created a model to further study Trisomy 21-associated hematopoietic disease. Moreover, flow cytometry findings suggest *STAG2* mutations may independently promote cell differentiation by increasing CD71 expression in a subset of cells. Taken together, these preliminary data suggest a model where *STAG2* mutations, in the context of Trisomy 21-associated GATA1s induction, may enhance TAM proliferation and differentiation toward AMKL.