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POSTER ABSTRACTS

636.MYELODYSPLASTIC SYNDROMES-BASIC AND TRANSLATIONAL

The Association of *SEPTIN6* Mutations with Pediatric Myelodysplasia

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Septin family proteins belong to the RAS-like GTPase superclass and are considered as a structural element of the cytoskeleton. They are evolutionarily conserved and are important in cytokinesis, polarity, cell cycle, vesicle trafficking, and exocytosis. They are implicated in T and B cell lymphomas; and acute myeloid leukemias, where they frequently act as fusion partners with the Mixed-Lineage Leukemia (MLL) gene. Previously, we described two non-syndromic patients with severe neutropenia which progressed to myelodysplastic syndrome (MDS) *(Renella et al. AJH, 2022, Mohamad et al. ASH, 2022)*. Next-generation sequencing revealed both patients (P1 and P2) to have novel X-linked germline mutations in codon 428 of the *SEPTIN6* gene (P1: SEPTIN6 c.1282T>C; P2: SEPTIN6 c.1282T>A). One of these patients (P1) had an additional somatic stop-gain mutation (P1: SEPTIN6 c.43C>T) of unclear function. Both patient germline mutations were predicted to add 9 amino acids to the C-terminal end creating a novel mutated SEPTIN6 protein. Since SEPTIN6 is located on the X-chromosome and expression of these mutated proteins occurred in males, no wild-type protein was present in the patient cells. To further explore the biological relevance of these mutations, single base-pair gene editing was used to introduce the (P1: T>C) mutation into normal male donor CD34+ cells. Morphological studies demonstrated that *SEPTIN6* edited cells had enlarged, multi-nucleated and dysplastic nuclei; and reduced clonogenic activity.

To further assess the effect of the T>C mutation on hematopoietic stem cell (HSC) function, the *SEPTIN6* edited cells were transplanted into immunocompromised NSGW mice. Analysis of peripheral blood demonstrated reduced chimerism in edited recipients (4.27% +/-3.4%) compared to controls (20.56% +/-6.2%, mean+/- SD, p= 0.0008) indicating a functional HSC defect upon mutation acquisition. Bulk mRNA sequencing demonstrated changes in ribosome, DNA replication, homologous recombination and mismatch repair pathways which imply DNA damage and cell cycle defects **(Figure 1)**.

To examine the potential dominant negative vs haploinsufficiency role of these mutations, we introduced the mutated *SEPTIN6* (P1: SEPTIN6 c.1282T>C) cDNA into wild type human male CD34+ cells. Morphological studies demonstrated enlarged cells with multi-nucleated and dysplastic nuclei. Moreover, clonogenic assays demonstrated that cells containing both the mutated and wild type protein had reduced number of colonies (17.7+/- 5.5) compared to controls (127+/- 13.7, mean+/- SD, p <0.0001). This indicates that the mutated SEPTIN6 protein has a dominant negative effect over the wild type protein.

We hypothesized that the somatic stop-gain mutation in the patient (P1) genome may mitigate the deleterious effects of the primary germline SEPTIN6 c.1282T>C mutation. To test this hypothesis, and determine the effect of each individual mutation, we created lentivirus vectors which contained a shmiR against the native *SEPTIN6* mRNA and also contained (a) the germline P1: SEPTIN6 c.1282T>C mutation (M2 mutation) alone, or (b) the germline M2 mutation and the somatic stop-gain mutation (P1: M1+M2 mutation) in tandem, or (c) germline P2: SEPTIN6 c.1282T>A (M3 mutation). The wild type *SEPTIN6* gene was reintroduced as a control. Clonogenic assays demonstrated that SEPTIN6 knockdown in human CD34+ cells caused no significant change in progenitor activity **(Table 1)**. Furthermore, introduction of the M2 or the M3 germline mutants in SEPTIN6 knocked down cells caused a reduction in the total number of colonies, especially the number of GM colonies with no change in the number of BFU-E colonies **(Table 1)**. This data validates our previous experiments performed with single base pair gene editing in human CD34+ cells. Interestingly, introduction of the stop-gain M1 mutation along with the M2 mutation (M1+M2) reversed the reduced clonogenic activity seen with expression of the M2 mutation **(Table 1)**. This indicates that the M1 mutation by introducing an early stop codon creates a SEPTIN6 knockdown effect and rescues hematopoietic function from the deleterious M2 mutation. Overall, our data demonstrates the acquisition of de novo gain of function mutations in

the *SEPTIN6* gene in myelopoiesis and suggests deleterious effects on engrafting hematopoietic stem cells. These mutations are associated with the development of MDS in two pediatric patients.

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Figure 1: Bulk mRNA Sequencing of Septin6 Edited Cells

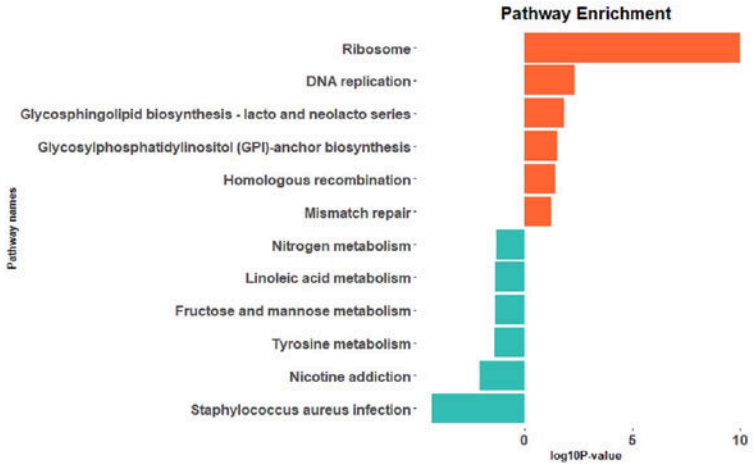

Table 1: Human clonogenic data.

Colony Number	Control		SEPTIN6 KD		SEPTIN6 KD + WT SEPTIN6		SEPTIN6 KD + M1 +M2 mutation		SEPTIN6 KD + M2 mutation		SEPTIN6 KD + M3 mutation	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Total	102.5	0.7	101.3	11.8	91.3	13.6	92.7	8.3	76	11	83	4.6
GM	65.5	2.1	56.3	3.2	52.7	5.5	55.3	6.1	38	6.6	42.7	4.5
BFU-E	36.5	2.1	42.7	8.7	37.7	9.9	36.3	4.7	37	4.6	37.7	1.5
GEMM	0.5	0.7	2.3	2.3	1	1.7	1	1	1	0	2.7	0.6

Figure 1

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