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# ORAL ABSTRACTS

## 101.RED CELLS AND ERYTHROPOIESIS, EXCLUDING IRON

### Whole-Genome CRISPR-Cas9 Screen Identifies *ZBTB7A* As a Potential Therapeutic Target for Cda-II

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Congenital dyserythropoietic anemia type II (CDA-II) is an autosomal recessive disease characterized by anemia, ineffective erythropoiesis, and increased bone marrow bi-nucleated erythroblasts. CDA-II is caused by loss-of-function mutations in *SEC23B*, which encodes a component of coat protein complex II (COP-II) vesicles/tubules that transport secretory proteins from the endoplasmic reticulum to the Golgi apparatus. Mammals express two SEC23 paralogs, SEC23A and SEC23B. We have previously shown that SEC23A is functionally interchangeable with SEC23B and that increased expression of SEC23A rescues the SEC23B-deficient erythroid differentiation defect observed in CDA-II.

Here, we utilized our recently generated clonal SEC23B deficient HUDEP-2 cell line to identify novel therapeutic targets for CDAII. SEC23B deficient HUDEP2 cells survive and grow normally when cultured as pro-erythroblasts in 'maintenance media'. However, upon culturing these cells in 'differentiation media', which results in semi-synchronous erythroid differentiation, differentiated *SEC23B*-null HUDEP2 cells exhibit reduced growth, impaired differentiation, increased bi-nucleated erythroid cells, and erythroid cell death, features of CDA-II.

To identify novel genes that play important roles in the pathophysiology of CDAII, we developed a functional genome-scale CRISPR knockout screen to define genes that when deleted, rescue the *SEC23B*-null erythroid differentiation defect. *SEC23B* null HUDEP2 cells cultured in maintenance media were infected with the hGeCKO-v2 lentiviral library at a multiplicity of infection (MOI) of ~0.3, allowing most transduced cells to receive 1 sgRNA (and Cas9) to knockout 1 gene only. Transduced cells were puromycin selected and passaged in expansion media for 14 days. *SEC23B*-null HUDEP2 cells were then differentiated for 10 days. Cells were harvested prior to differentiation (Day 0, D0), and differentiated (orthochromatic) cells were sorted at D10 of differentiation. Integrated sgRNAs were quantified by deep sequencing.

sgRNAs targeting *ZBTB7A* were amongst the most enriched in D10 compared to D0 erythroid cells. To validate these findings, we transduced *SEC23B*-null HUDEP2 cells with two independent and efficient *ZBTB7A*-targeting sgRNAs. We found that *ZBTB7A* deletion (using either of the 2 sgRNAs) rescued the lethality and differentiation defect of *SEC23B* null HUDEP2 cells undergoing differentiation. We next generated 7 clonal cell lines with combined deletion for *ZBTB7A* and *SEC23B* and confirmed that these cells exhibited normal growth and differentiation indistinguishable from wildtype HUDEP2 cells. The rescuing effect of targeting *ZBTB7A* was also validated in *SEC23B* mutated human CD34+ hematopoietic stem and progenitor cell culture.

Since SEC23A can functionally replace SEC23B in erythroid cells, we quantified the *SEC23A* mRNA level in *SEC23B*-null HUDEP2 cells deleted for *ZBTB7A*, by qRT-PCR. In early preliminary results, deletion of *ZBTB7A* resulted in a profound (~10 fold) increase in *SEC23A* mRNA expression, to a level predicted to be sufficient to rescue the *SEC23B* null erythroid differentiation defect. ChIP-seq analysis demonstrated that ZBTB7A occupies the *SEC23A* promoter in HUDEP2 cells.

Taken together, these data suggest that ZBTB7A represses *SEC23A* expression during erythroid maturation and that in the setting of *ZBTB7A* deletion, *SEC23A* expression is de-repressed, resulting in rescue of the CDA-II erythroid differentiation

defect. Genome editing of the ZBTB7A binding sites in the *SEC23A* promoter is ongoing in *SEC23B*-null HUDEP-2 cells. We will determine the impact of the latter editing on SEC23A expression and erythroid differentiation.

**Disclosures** No relevant conflicts of interest to declare.

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