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POSTER ABSTRACTS

201.GRANULOCYTES, MONOCYTES, AND MACROPHAGES

Neutrophil Elastase Catalytic Activity Regulates Granulopoiesis

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Heterozygous, autosomal dominantly inherited mutations in the gene, *ELANE*, encoding the neutrophil granule serine protease, neutrophil elastase, are the cause of cyclic neutropenia and the most common cause of severe congenital neutropenia. Many mutations throughout the protein occur in patients with congenital neutropenia, but they largely spare residues essential for enzymatic catalysis. Decades ago, neutrophil elastase was proposed to function as a chalone, in which its proteolytic activity feeds back to negatively regulate neutrophil production at steady state levels. To further determine if neutrophil elastase catalytic activity contributes to hematopoietic regulation and to evaluate how its mutations cause hereditary neutropenia, we have studied an induced pluripotent stem cell (iPSC) model of hematopoiesis, in which we have employed CRISPR/Cas9 to engineer a mutation not encountered in neutropenic patients. We inactivated neutrophil elastase's catalytic serine residue through substitution with alanine, generated CD34+ cells from iPSC, and directed their differentiation toward the granulocytic lineage. Compared to similarly edited isogenic controls in which the catalytic serine of neutrophil elastase remains intact, iPSC-derived CD34+ cells lacking the catalytic serine grew at a faster rate, failed to down-regulate *ELANE* expression while cyclically reactivating CD34 expression during granulocytic differentiation, produced greater quantities of neutrophils, and demonstrated residual progenitor cell populations in replating assays. Some, but not all, of these findings were reproducible upon treatment with small molecule inhibitors of neutrophil elastase. These observations suggest that neutrophil elastase's catalytic activity may help regulate granulopoiesis and could explain genotypic differences distinguishing cyclic neutropenia from severe congenital neutropenia.

Disclosures No relevant conflicts of interest to declare.

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