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Blood 142 (2023) 3828–3829

The 65th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

101.RED CELLS AND ERYTHROPOIESIS, EXCLUDING IRON

Whole-Genome CRISPR-Cas9 Screening Identifies Genes Required for Human and Mouse Erythroid Development

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Red blood cell (RBC) development is regulated by a few external signaling molecules which act to influence the ongoing intracellular processes responsible for producing erythrocytes. While much is known about the signaling molecules important for blood production, comparatively little is known about the genes required for successfully generating erythrocytes. Several genes have already been identified as essential for RBC development through loss-of-function studies in the mouse or in human erythroid cells *in vitro*. However, these studies have been limited in scope to genes that are already suspected or known to influence RBC production. Since there are ~10,000 genes expressed in erythroid cells, it is unclear how many of these genes are functionally required for erythroid development.

To define the repertoire of genes required for human erythroid development, we performed a genome-scale CRISPR knock-out screen in the human erythroid progenitor cell line HUDEP-2, using the GeCKOv2 lentiviral library, which delivers Cas9 and one of 6 sgRNAs targeting virtually every gene in the genome. Following viral transduction and cell recovery for 9 days, we collected HUDEP-2 cells prior to the onset of differentiation as well as differentiated orthochromatic erythroblasts (right prior to enucleation) using flow sorting, on the basis of CD49d expression (CD49d is downregulated in the final stages of erythroid differentiation).

As expected, sgRNAs targeting known erythroid essential genes (such as *GATA1* and *EPOR*) were depleted in HUDEP-2 cells compared to the sgRNA library. Notably, we also identified genes for which sgRNAs were depleted in differentiated versus undifferentiated erythroid cells, representing genes that are likely required for terminal erythroid differentiation (such as *ZFPM1*, *ALAS2*, etc..). This supports the utility of the screen to identify novel regulators of erythropoiesis.

Among highly ranked genes, *NHLRC2*, which was previously implicated in hemolytic anemia, was identified to be required in HUDEP-2 cells, suggesting that NHLRC2 is intrinsically required for human erythroid development. To validate the role of *NHLRC2* in human erythropoiesis, we down-regulated *NHLRC2* in primary human CD34+ hematopoietic stem and progenitor cells (HSPCs) undergoing erythroid differentiation, using one of 4 independent shRNAs. *NHLRC2* down-regulation resulted in impaired proliferation and differentiation of human erythroid cells *in vitro*.

To validate the results of the genome-scale screen, we generated a secondary library dedicated to examining genes that positively or negatively regulate the final stages of erythroid differentiation, while excluding common essential genes required for the survival of more than 90% of immortalized cell lines screened by the BROAD institute. The secondary screen yielded a list of over 500 genes found to influence erythroid differentiation (FDR<0.01). One of the novel genes, *VAC14*, was identified as a highly ranked gene in the secondary screen for erythroid differentiation. Validation experiments demonstrated that *VAC14* down-regulation using one of 3 independent shRNAs result in erythroid cell proliferation defects, both in HUDEP2 cells and in erythroid cells derived from primary human HSPCs.

Since germline loss of VAC14 is embryonic lethal in the mouse, to validate the role of VAC14 in erythropoiesis *in vivo*, we transplanted fetal livers from *Vac14* null mice (or wild-type [WT] control mice) into WT recipients and examined the VAC14

deficient hematopoietic compartment. Mice transplanted with *Vac14* null HSCs exhibited a profound reduction in absolute numbers of bone marrow erythroid cells with evidence of a block in erythroid maturation. Impaired bone marrow erythropoiesis was largely (but incompletely) compensated for by splenic extramedullary erythropoiesis. *Vac14* null hematopoietic cells exhibited pronounced cytoplasmic vacuolation, also affecting numerous stages of erythroid cells.
Altogether, we have performed an unbiased genome-scale CRISPR knock-out screen that identified (and validated) novel genes required in erythropoiesis.

Disclosures No relevant conflicts of interest to declare.

https://doi.org/10.1182/blood-2023-181929