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Blood 142 (2023) 3820–3821

The 65th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

101.RED CELLS AND ERYTHROPOIESIS, EXCLUDING IRON

A Genome Scale CRISPR Screen Identifies Novel Genes That Regulate Erythropoietin Production

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Erythropoietin (EPO) is a plasma glycoprotein that binds bone marrow erythroid progenitors, stimulating their proliferation and differentiation. EPO is secreted into the circulation by hepatocytes and specialized kidney peritubular fibroblasts, which contribute ~20% and ~80% of the total plasma EPO level, respectively. EPO production is induced during hypoxia, due to enhanced stability of the transcription factor hypoxia-inducible factor (HIF). However, increased HIF activity results in enhanced expression of angiogenesis and oncogenesis promoting genes, raising concerns about clinical strategies that promote EPO production via increasing HIF levels.

To define novel regulators of EPO production, we developed a genome-wide functional screen that provides a quantifiable and selectable readout of EPO production. In order to perform such a screen, we generated a reporter HEP3B cell line with homozygous insertion of coding sequences for p2A and eGFP at the endogenous EPO locus. This HEP3B EPO-p2A-eGFP (HEG) cell line expresses equivalent levels of EPO and eGFP proteins, both translated from the same mRNA molecule. Treatment of HEG cells with the HIF stabilizing molecule DMOG results in increased EPO expression, which is abolished by deletion of the hypoxia-responsive-element in the *EPO* locus. These findings demonstrate the utility of this reporter cell line to study the regulation of EPO production.

To identify novel genes that regulate EPO production, we mutagenized the HEG cell line with the GeCKO-v2 CRISPR/Cas9 knock-out library, which delivers a puromycin resistance cassette, SpCas9, and a pooled collection of 123,411 single guide RNAs (sgRNAs) that include 6 independent sgRNAs targeting every human gene. Transduction was performed at a low multiplicity of infection (MOI ~0.3), so that most infected cells receive 1 sgRNA to delete 1 gene only. Puromycin selection was applied from days 1-4 post-transduction and cells were subsequently recovered for 8 days. Cells were then treated with DMOG for 24 hours or left untreated. Cells with increased (top ~10%) or decreased (bottom ~10%) eGFP fluorescence were isolated. This screen was done in biological triplicates. Genes for which sgRNAs are enriched/depleted in eGFP high versus eGFP low DMOG-treated cells likely impact EPO expression independently of the HIF pathway. In contrast, genes for which sgRNAs are enriched/depleted in eGFP high versus eGFP low untreated cells may impact EPO expression in either a HIF dependent or independent manner.

As expected, sgRNAs targeting *VHL* were found to be among the most significantly enriched sgRNAs in eGFP-high versus eGFP-low cells under no treatment conditions. Also as anticipated, sgRNAs targeting genes known to promote EPO produc-

tion (*HNF4A*, *WT1*, *RXRA*, and *CREBBP*) were enriched in eGFP-low versus eGFP-high cells under either DMOG-treated or untreated conditions.

To refine our findings, we generated a custom library targeting the top 1,200 candidate regulators of EPO production nominated by the genome-scale screen. Using this library, we performed a secondary CRISPR screen (in biological triplicates) as above. Owning to the 10-fold smaller size of the custom library, the secondary screen was performed with average coverage of >150x throughout the entire screen (including the sorted cell populations). Indeed, the statistical confidence obtained with the secondary screen was superior than that achieved in the genome scale screen.

Using a false discovery rate (FDR) cutoff of 1%, gene-level analysis identified 100 and 79 candidate genes whose targeting was associated with increased or decreased EPO production respectively, under either untreated or DMOG-treated conditions. A high degree of concordance was observed for regulators of EPO expression between both screens, performed with and without DMOG treatment.

To identify genes that regulate EPO production independent of HIF, we evaluated several of the top genes for which sgRNAs were enriched in eGFP high vs. eGFP low cells treated with DMOG. Using 3 independent sgRNAs targeting each of these candidates, we found 2 genes (*ZNF574* and *RBM18*), that when deleted, result in increased EPO mRNA, even following DMOG treatment. Additional validation experiments as well as studies aimed to define the role of these 2 genes in the regulation of EPO production are ongoing.

Disclosures No relevant conflicts of interest to declare.

https://doi.org/10.1182/blood-2023-182427