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POSTER ABSTRACTS

101.RED CELLS AND ERYTHROPOIESIS, EXCLUDING IRON

Identification of Chromatin Regulators Required for Enucleation

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Erythropoiesis involves several distinct steps as precursor cells to differentiate and mature into erythrocytes which follows differentiation to proerythroblasts, transformation into basophilic, polychromatic, and orthochromatic erythroblasts, followed by enucleation to form reticulocytes and erythrocytes. Indeed, erythropoiesis and enucleation are complex processes that involve the coordinated action of multiple regulators. Various transcription factors, signaling pathways, and epigenetic modifiers play significant roles in controlling gene expression, cell differentiation, and cellular events during erythropoiesis. As an example, BCL11A is necessary for nucleus expulsion and the progression of erythropoiesis. Similarly, other transcription factors, histone deacetylases, DNA methylation enzymes, and chromatin remodeling complexes also contribute to the enucleation process. However, the exact role of chromatin regulators and how they are involved in the enucleation process is still not fully understood.

To identify novel chromatin factors that regulate both enucleation and proliferation of erythroid cells, we conducted a pooled epigenome-wide CRISPR-Cas9 knockout screen using the human erythroid cell line BEL-A. The epigenetic knockout library consists of 719 genes associated with chromatin-related pathways and 35 essential genes, which include those encoding ribosomal proteins. Each gene is targeted by 10 single guide RNAs (sgRNAs). The primary objective of this screen was to determine essential chromatin regulators that play a critical role in the process of enucleation. To achieve this, we planned the screening groups as followed: (i) cells collected immediately after antibiotic selection (time zero), (ii) undifferentiated, expanded cells grown for 13 days, (iii) expelled nuclei (pyrenocytes), and (iv) orthochromatic erythroblasts that have not undergone enucleation. To isolate the distinct cellular stages mentioned above, we utilized fluorescence-activated cell sorting (FACS). The pyrenocyte group was compared with all other groups to identify depleted gRNAs, which represent factors that are necessary for enucleation. Setd8, which is an important epigenetic factor that is required for enucleation was determined to be depleted only in pyrenocytes (β score -0,433), validating our screen approach.

In addition, we identified more than 15 other genes that showed even greater depletion than Setd8, and could be essential for enucleation. Among the identified genes, EP300, PCGF1, Setd1B, CDY2B, and TLK2 were found to be the most depleted in pyrenocytes. Additionally, TRIM28, KAT5, and EXOSC7 were identified as enriched genes in the study. On the other hand, we identified a group of genes that exhibited significant depletion in pyrenocytes compared to orthochromatic erythroblasts. Among these genes, UHRF1, DPF2, KEAP1, and TAF10 were found to be most depleted.To better understand the role of KEAP1 in enucleation, KEAP1 knockout (KO) BEL-A cells were generated. The enucleation ratio of the KEAP1 KO cells decreased significantly, and their survival also dramatically decreased after differentiation on day 12 compared to the wild-type cells. Live cell imaging of enucleation of the KEAP1 KO cells further confirmed the decrease of the enucleation rate.

The significant decrease of enucleation observed in KEAP1 KO cells has triggered questions about the specific pathways and processes required for enucleation. Given that KEAP1 is the main regulator of NRF2, our future studies will mainly focus on understanding the importance of oxidative stress in enucleation.

Disclosures No relevant conflicts of interest to declare.

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