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POSTER ABSTRACTS

101.RED CELLS AND ERYTHROPOIESIS, EXCLUDING IRON

Rapid Degradation of mDia2 Protein during Terminal Erythropoiesis Via an In Vivo Aid System: An Alternative Approach for Loss-of-Function Studies

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Loss-of-function manipulations are crucial methods for studying gene functions. Despite the availability of many chemical-induced genetic manipulation techniques, there are limitations associated with these technologies. Tamoxifen-induced conditional gene expression, gene knockdown, or cell tracking represent some of the most common genetic manipulations employed in mice. However, tamoxifen-induced gene or Cre expression in bone marrow cell populations is relatively weak, affecting only a low proportion of cells. Similar observations have been made with the in vivo doxycycline-inducible system. Given that inflammation could affect the dispersion of bone marrow hematopoietic cells, an inducible system based on the MX1 promoter and interferon-alpha is not suitable for long-term protein knockdown. In addition, the above-mentioned methods are not able to achieve rapid loss-of-function because it is largely determined by the half-life of the transcriptional and translational products of the targeted gene. Furthermore, none of these technologies could achieve reversible depletion of the protein of interest. Therefore, there is a need for enhanced techniques for inducible and reversible loss-of-function gene manipulations in the field of hematopoiesis. The capacity for quick, targeted protein elimination is particularly necessary when researching the functions of proteins that play roles in swift biological processes.

The Auxin-inducible degron (AID) system is a chemically inducible tool, widely employed for targeted protein degradation to study protein functions in cultured mammalian cells. It holds significant potential as a powerful tool for studying protein functions in vivo within the bone marrow. The AID system comprises three components: the auxin signal, AID tag, and auxin receptor F-box proteins (AFBs). When an AID tag is fused to a protein of interest, the AFB proteins recognize the tag and, in the presence of the auxin signal, induce the protein's degradation via the proteasome. Additionally, due to its small molecular weight, auxin can potentially penetrate the bone marrow barrier and achieve the working concentration in the bone marrow, facilitating protein degradation.

In this study, we aim to investigate the role of mDia2 in terminal erythropoiesis using AID system. The mDia family proteins are formin proteins that catalyze the assembly of F-actin. The loss of function of mDia2 achieved by Cre-flox system leads to deficiency in enucleation and anemia. We hypothesize that the mDia2 loss of function could be compensated by upregulated mDia1 in the Cre-flox mediated knockout system, and a rapid loss of mDia2 protein could lead to more severe phenotypes in vivo and ex-vivo. We knocked in miniIAA7-eGFP to the end of the last exon of mDia2 gene to create an endogenous tagged mDia2 protein in C57/B6 mice. We also created an AFB protein transgenic mouse by knocking in the atAFB2 protein coding gene at the ROSA26 locus driven by the Vav1 promoter. After crossing the mDia2-miniIAA7-eGFP transgenic mice with Vav1-atAFB2 mice, we obtained mDia2-miniIAA7-eGFP and Vav1-atAFB2 double homozygous mice.

We administered auxin via intraperitoneal (IP) injection at a concentration of 300 mg/kg and collected total bone marrow cells four hours post-injection. Quantification of the GFP signal through flow cytometry revealed a significant reduction following auxin administration compared to the control group. This protein degradation was further confirmed with Western blotting assays.

To understand how an immediate loss of mDia2 influences terminal erythropoiesis, we isolated lineage-negative cells from auxin-administered mice and cultured them in an EPO-containing medium with 10 μM auxin for 48 hours. Compared to lineage-negative cells from VavCre-mDia2f/f mice, auxin-induced mDia2 degradation resulted in a significantly lower enucleation rate. Notably, auxin administration had no observable effects on either WT mice or atAFB2 homozygous mice. This suggests that rapid mDia2 loss, induced by the AID system, permits a better understanding of the targeted protein's function by avoiding potential compensatory effects from mDia1 during terminal enucleation.

In conclusion, our established in vivo AID system enables rapid targeted protein degradation and serves as a powerful tool for studying protein functions potentially obscured by compensatory effects during erythropoiesis.

Disclosures No relevant conflicts of interest to declare.

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