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Instantaneous Manipulation: Dynamic Regulation of Eomes in Early Mouse Embryonic Development

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The paper "A degron-based approach to manipulate Eomes functions in the context of the developing mouse embryo" published in the journal *Proceedings of National Academy of Sciences of the United States of America* on October 31, 2023, was written by Alexandra M. Bisia, Ita Costello, Maria-Eleni Xypolita, Luke TG Harland, etc., from the Sir William Dunn School of Pathology, University of Oxford, Oxford, UK. The study explored the function and role of this gene in different developmental stages of mouse embryos by constructing Eomes gene variants containing a degradation fusion tag (dTAG). By using the dTAG system, researchers can quickly and specifically induce the degradation of Eomes protein through small molecule intervention in both in vitro and in vivo environments, thereby revealing the key role of Eomes in embryonic development, and also demonstrating the application potential and flexibility of dTAG technology in studying gene function.

1 Interpretation of Experimental Data

The study used the degradation fusion tag (dTAG) system to create a new Eomes gene editing tool to quickly and specifically manipulate the expression of Eomes protein, revealing its key functions in various stages of mouse embryonic development. By applying the dTAG system in mouse embryonic stem cells and mice, the research team deeply explored the dynamic expression and function of Eomes in various cell lineages. The results showed that the dTAG system can quickly and reversibly degrade Eomes protein, providing a powerful tool for studying the spatiotemporal specific role of Eomes in embryonic development and cell lineage differentiation.

Figure 1 presents the gene-editing strategy using CRISPR-Cas9 technology to construct a degradable variant of the Eomes protein ($Eomes^{deg}$). Researchers used crRNA targeting the Eomes gene along with an ssDNA repair template containing a degradation tag (Deg), a self-cleaving 2A peptide, and an mCherry reporter gene, successfully creating the $Eomes^{deg}$ allele. This modification allows for tracking of Eomes protein expression via the mCherry label. In the experimental protocol, embryoid bodies (EBs) were formed under the induction of 2i/LIF medium and further promoted to differentiate into mesodermal/HE markers by adding factors such as BMP4, ActA, and VEGF. Western blot results show that the Eomes protein can be degraded within one hour of treatment with dTAG-13. Flow cytometry results confirmed that $Eomes^{deg/deg}$ EBs treated with dTAG-13 lost Eomes protein expression. These results validate the effectiveness of the dTAG-13 mediated rapid degradation of Eomes protein, providing a powerful tool for in-depth study of Eomes functions during embryonic development.

Figure 3 presents the experimental design and results of administering dTAG treatment to mouse embryos at 6.5 days post-coitum (dpc) via intraperitoneal injection. The experimental flow diagram (Figure 3A) illustrates the method of dTAG treatment, while Figure 3B uses immuno fluorescence staining to show the expression and degradation of Eomes (green) and Foxa2 (red) in the $Eomes^{deg/deg}$ homozygous embryos after treatment. DAPI (blue) stains the nuclei, revealing heterogeneity in the degree of Eomes protein degradation across different regions. Notably, embryos treated with dTAG-13 show Eomes-positive cells in the posterior region of the primitive streak, whereas embryos treated with dTAGV-1 still express Eomes in the extraembryonic ectoderm, the extraembryonic visceral endoderm (VE), and the primitive streak. These results indicate that dTAG-mediated

protein degradation exhibits regional differences in vivo, highlighting the potential of this technique in studying the function of Eomes during embryonic development and the technical challenges associated with its application.

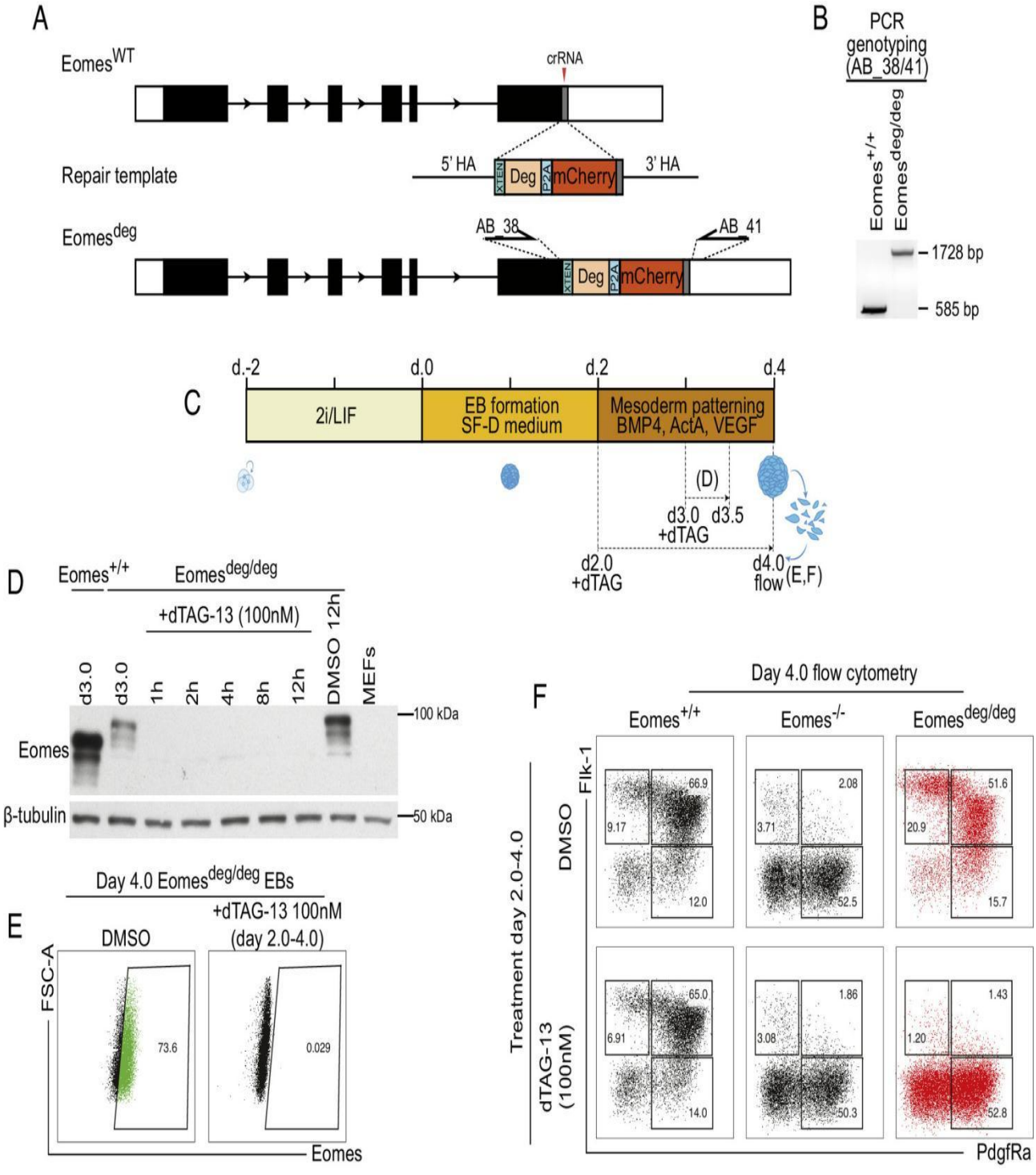

Figure 1 Generation of an Eomes^{deg} allele using CRISPR-Cas9 and functional analysis in vitro during HE differentiation

Figure 5 evaluates the recovery process of Eomes protein in Eomes $^{deg/deg}$ embryos and embryoid bodies (EBs) after washing with dTAG-13 or dTAGV-1 and validates the expression of the mCherry reporter gene. Figure 5A illustrates the processing and cleaning steps for small molecule dTAG. Figure 5B shows Eomes (green) and Foxa2 (red) staining in E6.5 embryos treated with DMSO, dTAG-13, or dTAGV-1, showing Eomes expression in the proximal posterior region after two hours of washing and reculture. Ectodermal/primitive bar area restoration. Figure 5C shows the staining of Eomes (green) and Nanog (red) in HE EBs after dTAG-13 treatment on the third

day, evaluating the dynamic process of Eomes recovery. After 1 hour and 2 hours of dTAG-13 treatment, Eomes staining was undetectable; 1 hour after washing, Eomes nuclear staining was obvious, revealing the rapid recovery of Eomes protein. Figure 5D shows Eomes (green) and RFP (red) staining in E7.5 Eomes$^{deg/+}$ embryos. RFP detects the expression of the mCherry reporter gene. Figure 5E analyzes the expression of mCherry in E7.5 dissociated Eomes$^{deg/deg}$ embryos by flow cytometry. About 70% of the cells express mCherry.

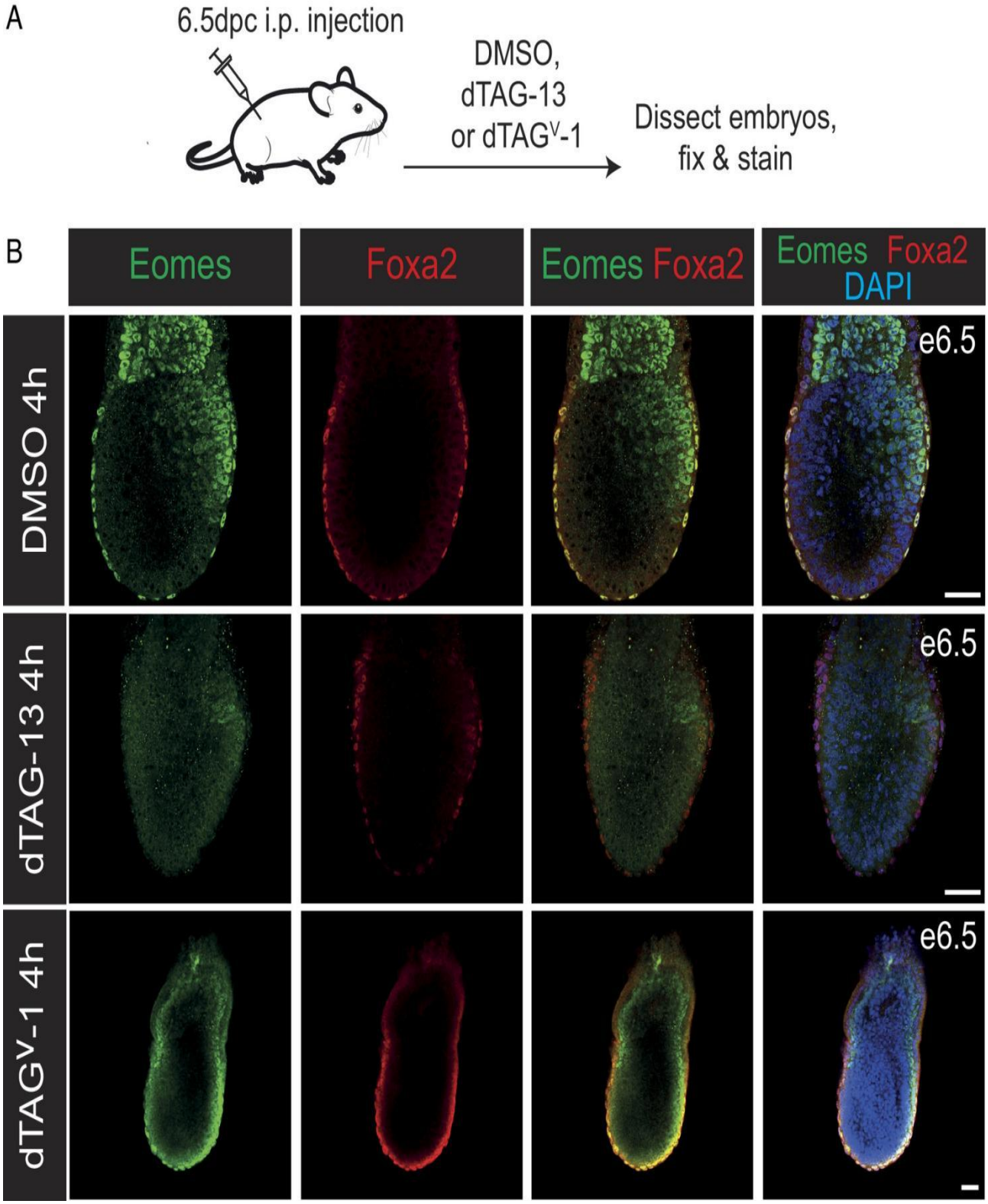

Figure 3 dTAG treatment of in utero embryos at 6.5dpc

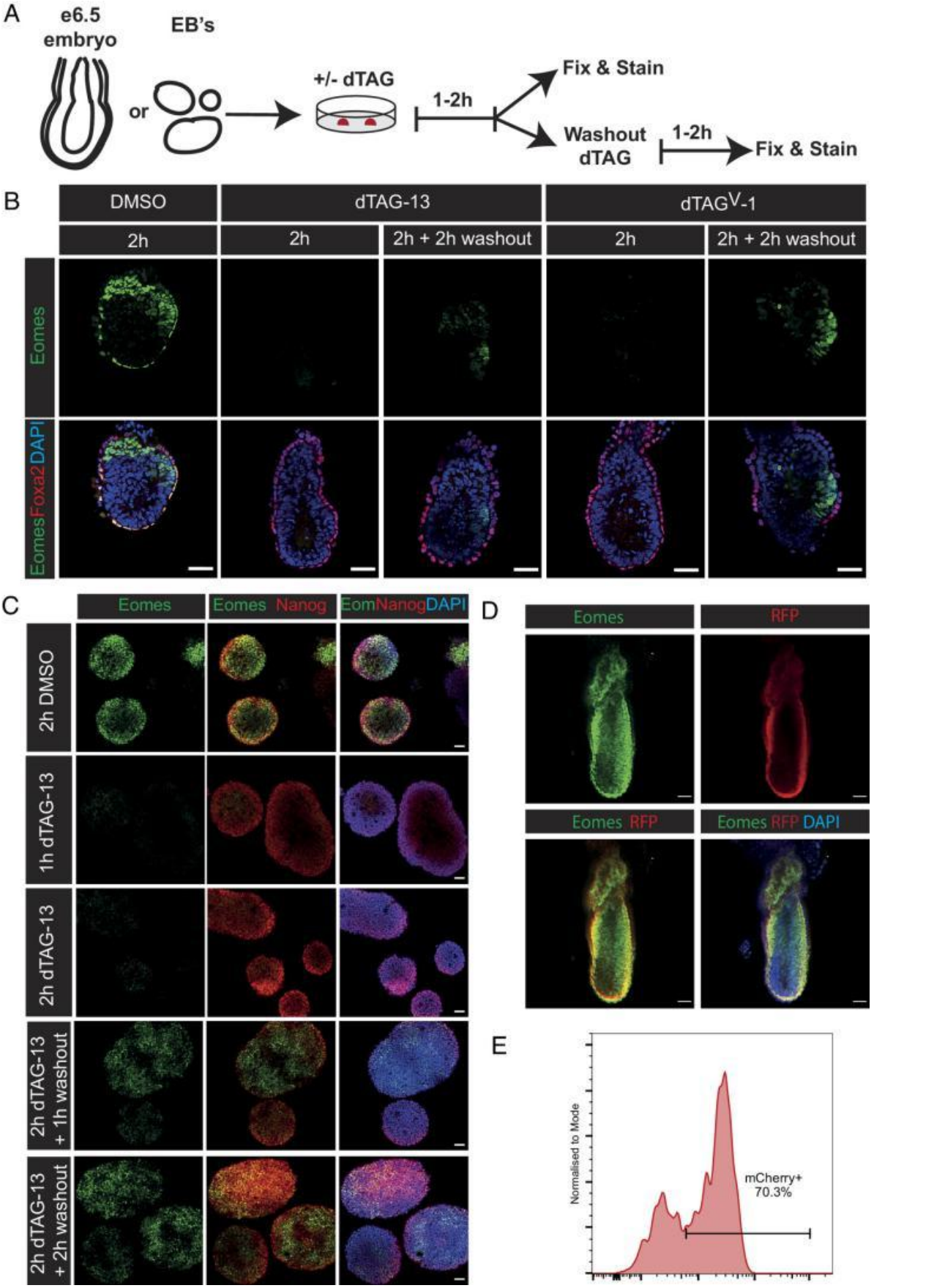

Figure 5 Ex vivo treatment of Eomes$^{deg/deg}$ embryos and EBs to assess the time-course of Eomes recovery after dTAG-13 or dTAGV-1 washout and validation of the mCherry reporter

2 Insights of Research Findings

This study successfully applied the dTAG system to a mouse embryo model, achieving rapid and reversible control of Eomes protein, thus demonstrating the potential of this technology for controlling gene expression at the whole-organism level. Eomes, a key T-box transcription factor, plays a crucial role at multiple stages of embryonic development. By combining in vitro and in vivo experiments, this study extensively explored the functions of Eomes across different cell types and its temporally and spatially specific expression during embryonic development. In vitro experiments involved the introduction of dTAG labels at the Eomes site, creating an embryonic stem cell line that could rapidly degrade Eomes protein upon the application of the small molecule dTAG-13. The experiments showed that Eomes protein could be almost completely lost within one hour and quickly restored after the removal of dTAG-13, providing a powerful tool for studying its role in cell development. In vivo experiments involved injecting pregnant mice with dTAG-13, attempting to degrade Eomes protein specifically at different stages of embryonic development. Results revealed that the efficiency of Eomes protein degradation varied greatly among tissues, particularly during early embryonic stages, where its degradation significantly affected implantation and further development. This underscores the critical role of Eomes in determining early cell fate and reveals its complex regulatory mechanisms in cell differentiation and tissue formation. These findings not only showcase the potential of the dTAG system for studying key developmental genes with strict temporal and spatial expression but also highlight the importance of precise genetic manipulation techniques in understanding complex biological processes, offering new possibilities for future disease treatment research. Through this method, researchers can delve deeper into the roles of key transcription factors like Eomes in various biological processes.

3 Evaluation of the Research

This study used dTAG technology to introduce an innovative tool that can rapidly and reversibly manipulate the expression of target proteins. Compared with traditional genetic knockout methods, dTAG operations are more flexible and faster, and are particularly suitable for studying genes that are transiently expressed during development. Studies have shown that the dTAG system can efficiently degrade specific proteins in an in vitro environment, but there are differences in the efficiency and consistency of protein degradation in in vivo experiments. This may be affected by multiple factors such as the expression level of the target protein in different tissues, differences in cell types, drug bioavailability, and tissue permeability. In addition, protective structures in the early embryo may hinder the penetration of small molecules, thereby affecting experimental results. Despite the challenges, the dTAG system provides a unique mechanism to deeply explore the dynamic regulation of proteins and their role in development. In the future, optimizing the design of dTAG molecules will further expand their potential for application in disease models and treatments.

4 Concluding Remarks

Through the application of the dTAG system in this study, the researchers successfully degraded the Eomes protein specifically at different developmental stages, revealing its key role in early embryonic development in mice. As a T-box transcription factor, Eomes is particularly important in embryonic gastrulation, cell lineage determination, and organ development. The use of the dTAG system allows for rapid degrading of Eomes at specific times and cell types, thereby observing the dynamic effects of its loss of function on embryonic development. This method is superior to traditional gene knockout technology because it can observe the loss of specific gene function without affecting the overall survival of the embryo. This study provides a new perspective for understanding how Eomes regulates cell fate at different developmental stages, and demonstrates the potential of dTAG technology in disease model construction and the development of new therapeutic strategies.

5 Access Original Paper

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