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Potential Pitfalls of the *Mx1-Cre* System: Implications for Experimental Modeling of Normal and Malignant Hematopoiesis

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SUMMARY

Conditional knockout mice are commonly used to study the function of specific genes in hematopoiesis. Different promoters that drive *Cre* expression have been utilized, with the interferon-inducible *Mx1-Cre* still being the most commonly used "deleter strain" in experimental hematology. However, different pitfalls associated with this system could lead to misinterpretation in functional studies. We present here two of these issues related to the use of *Mx1-Cre*: first, a high spontaneous recombination rate when applying commonly used techniques in experimental hematology, and second, undesired short-term consequences of the use of polyinosinic:polycytidylic acid, including changes in cellular phenotypes that, however, resolve within days. Our studies emphasize therefore that proper controls are crucial when modeling gene deletion using the *Mx1-Cre* transgene.

INTRODUCTION

Conditional knockout (cKO) mice based on *Cre*-mediated excision (Jiang and Gridley, 1997) are frequently used when studying normal and malignant hematopoiesis. Most cKO models are generated by flanking a gene segment of interest with *loxP* sites, which allows for its removal in a cell- or tissue-specific manner by *Cre* recombinase. The *Mx1-Cre* model (Kuhn et al., 1995) represents the most commonly used "deleter strain" in experimental hematology. In this strain, the Mx dynamin-like GTPase 1 (*Mx1*) promoter is activated in an interferon-dependent manner following injection of polyinosinic:polycytidylic acid (pIpC), which results in downstream expression of *Cre* recombinase. Despite the wide use of the *Mx1-Cre* strain, caution has to be entertained due to caveats that have not been well described. These include spontaneous recombination and undesired side effects caused by the pIpC itself.

In experimental hematology, bone marrow (BM) transplantation represents a key approach in the investigation of the role of specific genes. Most often, this is performed with cells obtained from cKO mice. We show here that the transplantation assay and expression of an activated receptor tyrosine kinase (FLT-3^{ITD}) can trigger spontaneous *Mx1-Cre*-driven deletion of floxed genes, which substantially exceeded the 2%–3% originally reported (Kemp et al., 2004; Kuhn et al., 1995; Mupo et al., 2013). In addition, we observed transient changes in phenotype and frequency of hematopoietic stem and progenitor cells (HSPCs) after pIpC injection, which could lead to incorrect identification of subsets of cells in short-term studies. Finally, we propose a potential alternative strategy for gene deletion using an ex vivo Tat-Cre-recombination approach. Awareness of these shortcomings has high relevance for hematopoietic research because it can aid in experimental design and avoid potential errors in the interpretation of results.

RESULTS

Transplantation Induces Uncontrolled Recombination in Hematopoietic Cells from *Mx1-Cre* Mice

To study the influence of HIF-1α in the initiation of leukemia, we retrovirally introduced different oncogenes into *Mx1-Cre*; *Hif-1α*$^{flox/flox}$ cKIT^{+} cells and transplanted them into wild-type (WT) recipients (Velasco-Hernandez et al., 2014). Surprisingly, we observed an unexpectedly high deletion frequency of the floxed gene prior to pIpC injection, ranging between 30% and 50% (Figure 1A).

To identify experimental procedures that contribute to the spontaneous *Mx1-Cre* activation, we crossed Rosa26-Lox-Stop-Lox-YFP (YFP^{LSL}) reporter mice (Srinivas et al., 2001) with the *Mx1-Cre* mice. HSPCs from these mice were next subjected to a routine protocol for transduction/transplantation experiments, using retroviral vectors encoding fluorescence proteins (Figures 1B–1G). Isolated unmanipulated cells from donor mice displayed a spontaneous recombination close to 20% (Figures 1C, 1D, 1E, 1H, and S1). Analyses after transplantation indicated that

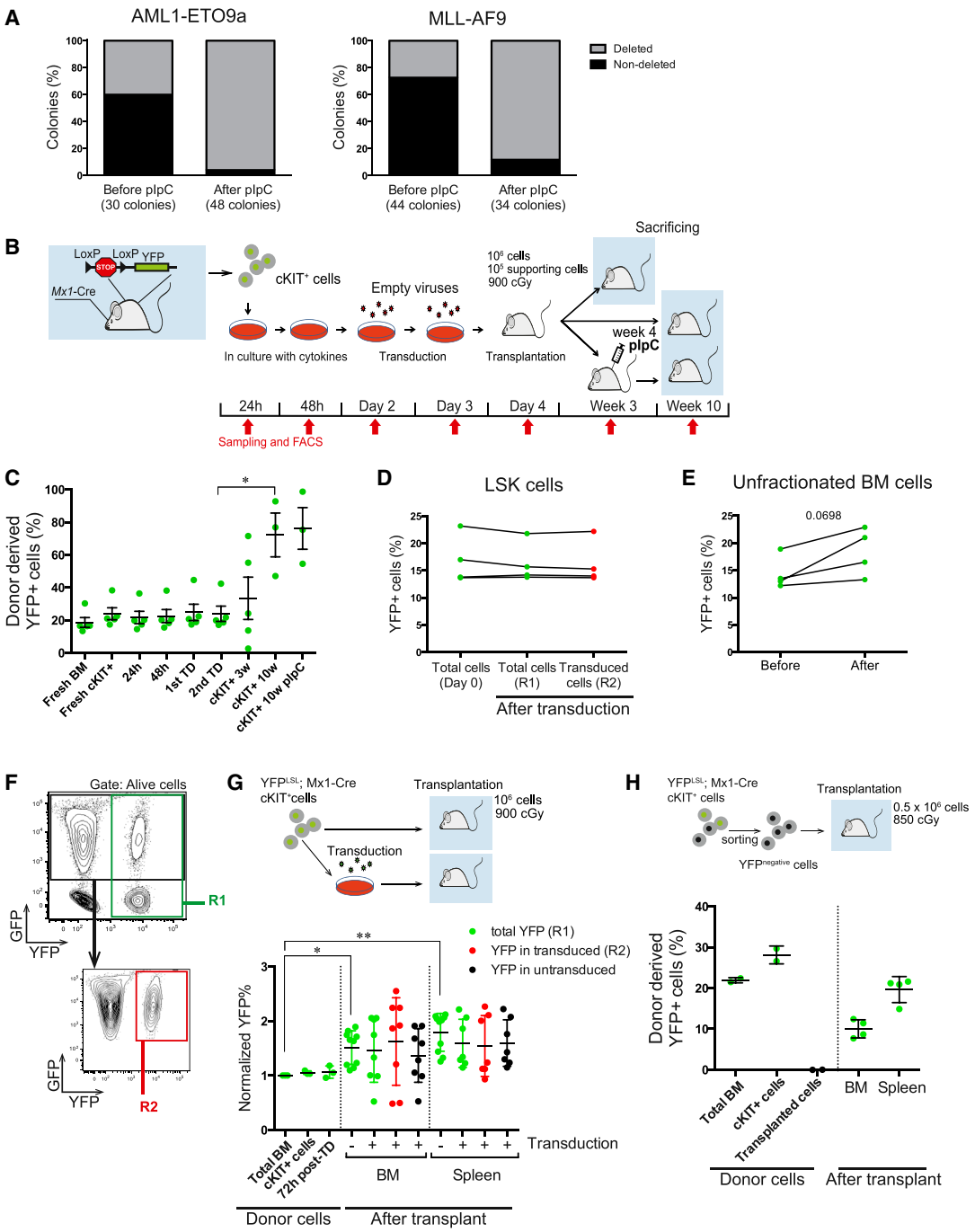

Figure 1. Spontaneous Deletion of Floxed Genes in Hematopoietic Cells from *Mx1-Cre* Mice after Transplantation

(A) Frequency of HIF-1α-deleted colonies from peripheral blood cells derived from animals transplanted with transduced *Mx1-Cre; Hif-1α*$^{flox/flox}$ HSPCs before (week 3 post-transplantation) and after pIpC injection (pIpC injection at week 4 and analysis at week 7). Plots show two independent experiments, using either AML1-ETO9a or MLL-AF9 expressing donor cells.

(B) Experimental setup for analysis of spontaneous deletion in a commonly used transduction/transplantation protocol. Donor cells were collected from five separate animals.

(C) Variation in frequency of recombined cells (YFP+) along the different steps of the protocol described in (B). Percentage of recombined cells is calculated within the donor-derived population after the transplantation step. *p < 0.05.

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