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Inactivation of Klf5 by zinc finger nuclease downregulates expression of pluripotent genes and attenuates colony formation in embryonic stem cells

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Abstract Recent studies suggest that Klf5 is required to maintain embryonic stem (ES) cells in an undifferentiated state. However, whether Klf5 can be inactivated by novel fusion technology of zinc finger nucleases (ZFN) has never before been examined. Therefore, we used ZFN technology to target the Klf5 gene in mouse ES cells, and examined the effects of the Klf5 gene on the expression of pluripotency-related genes, Oct3/4, Nanog, and Sox2 and on the self-renewal of ES cells. In Klf5–ZFN-transfected cells, expression of the Klf5 mRNA was downregulated by ~80 % compared to the control. Furthermore, expression of the Oct3/4 and Nanog mRNAs was significantly decreased in the Klf5–ZFN-targeted cells. RT-PCR analysis, however, showed no significant change in the level of Sox2 mRNA, but a decreased trend was evident in the Klf5–ZFN-targeted cells. Moreover, we observed the spontaneous differentiation of Klf5–ZFN-transfected cells and quantitative analysis revealed a significant decrease in colony formation in Klf5–ZFN-transfected cells. In conclusion, our data suggest that ZFN methodology is an effective approach to target the Klf5 gene and that Klf5 plays an important role in the maintenance of ES cell self-renewal.

Keywords Self-renewal · Differentiation · Zinc finger nucleases · Klf5 · ES cells

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Introduction

Embryonic stem (ES) cells, derived from the inner cell mass of early embryos, have the potential to self-renew indefinitely and differentiate into any cell type of all three primary germ layers [1, 2]. Pluripotency of ES cells is maintained by a highly interconnected regulatory network involving several key transcriptional factors, such as Oct3/4, Nanog, and Sox2 [3–5]. Recent studies suggest that the molecular mechanisms of ES cell self-renewal are more complex than previously anticipated [6]. Therefore, it is conceivable to employ novel technologies to further explore the underlying mechanisms of self-renewal involving various factors.

In this regard, Kruppel-like factors (Klfs) have recently received much attention [7]. Klfs are a subfamily of the zinc finger class of transcriptional factors, characterized by their three Cys2 His2 zinc fingers located at the C-terminus, separated by a highly conserved H/C link [7, 8]. So far, over 20 Klf family members that play important roles in many biological processes, including cell growth, proliferation, differentiation, apoptosis, and survival have been identified [9–11]. Current studies have shed light on some of the Klf family members which may play a crucial role in the maintenance of self-renewal of ES cells [12–14]. These family members include Klf2, 4, and 5. However, their exact role in the maintenance of the undifferentiated status of ES cells remains to be further elucidated. Klf4 has been used to generate various iPS cells and implicated in the self-renewal process [14]. Moreover, the role of Klf5 in ES cell pluripotency is still being deduced and not fully understood. Homozygous disruption of the Klf5 gene at a very early stage of embryonic development is lethal [15]. The Klf5 null embryos can neither develop beyond the blastocyst stage nor produce any ES cell line, suggesting