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GSK3 inhibition, but not epigenetic remodeling, mediates efficient derivation of germline embryonic stem cells from nonobese diabetic mice

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ABSTRACT

The nonobese diabetic (NOD) mouse strain is a predominant animal model of type 1 diabetes. However, this mouse strain is considered to be non-permissive for embryonic stem cell (ESC) derivation using conventional methods. We examined small molecule inhibition of glycogen synthase kinase 3 (GSK3) to block spontaneous cell differentiation and promote pluripotency persistence. Here we show a single pharmacological GSK3 inhibitor, 6-bromoindirubin-3′-oxime (BIO), in combination with leukemia inhibition factor (LIF), promoted generation of stable NOD ESC lines at > 80% efficiency. Significantly, expansion of the established NOD ESC lines no longer required treatment with BIO. These NOD ESC lines contributed to chimeric mice and transmitted to germline progeny that spontaneously developed diabetes. By contrast, 5-aza-2′-deoxycytidine (AZA), a small molecule inhibitor of DNA methylation, and trichostatin A (TSA) and valproic acid (VPA), small molecule inhibitors of histone deacetylase, could not promote generation of NOD ESCs by epigenetic remodeling. These combined findings provide strategic insights for imposing pluripotency in cells isolated from a non-permissive strain.

1. Introduction

The NOD mouse strain has been widely used for investigating autoimmune disease and immune tolerance mechanisms (Pearson et al., 2016). An advantage of this strain is that it develops spontaneous autoimmune diabetes with many similarities to human type 1 diabetes (T1D) (Anderson and Bluestone, 2005; Wicker et al., 2005). These include complex genetic susceptibility, the presence of autoreactive lymphocytes and shared disease pathology (Driver et al., 2011; Ridgway et al., 2008). ESC technologies provide a proven strategy to generate genetically altered cell lines, differentiated cells and mice for studying molecular and cellular pathways in biological processes and disease pathogenesis (Gerlai, 2016). However, derivation of useful germline competent ESCs from NOD mice has proved difficult using conventional methods (Brook et al., 2003; Nagafuchi et al., 1999).

The discovery of ground-state culture conditions revealed that ESC derivation and maintenance are enabled by inhibition of GSK3 and/or ERK1/2, which suppresses residual differentiation (Sato et al., 2004; Umehara et al., 2007; Ying et al., 2008). Subsequently, a few groups have generated NOD ESCs using different combinations of inhibitors: Hanna et al. used inhibitors of GSK3 and/or ERK1/2, but resultant ESCs required continuous presence of the inhibitors for stable expansion (Hanna et al., 2009); Nichols et al. also used two inhibitors (2i, one GSK3 inhibitor and one ERK1/2 inhibitor) (Nichols et al., 2009); whereas Ohta et al. reported using 3i (one GSK3 inhibitor and two ERK1/2 inhibitors) (Ohta et al., 2009). Previous studies have also shown that epigenetic remodeling increases the success rate of somatic cell nuclear transfer and generating induced pluripotent stem cells (iPSCs) and ESCs (Huangfu et al., 2008; Kim et al., 2009; Kishigami et al., 2007; Mikkelsen et al., 2008). Nonetheless, it has not been reported if epigenetic remodeling can improve the derivation of stable NOD ESCs. In this study, we compared the effects of inhibiting GSK3 and epigenetic modifiers for establishing NOD ESC that do not require additional inhibitors for expansion and eventual derivation of NOD mice that develop spontaneous autoimmune diabetes.

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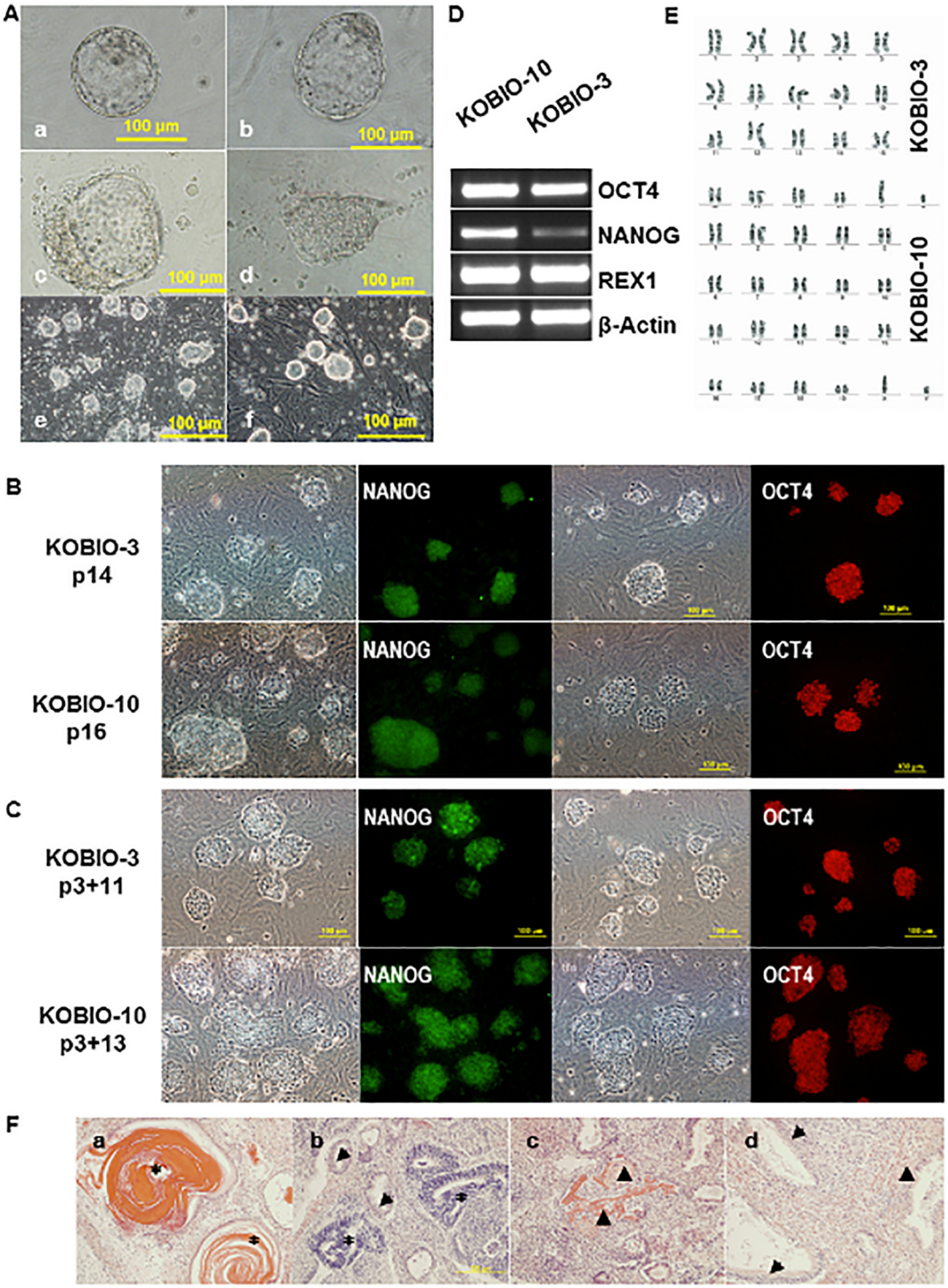

Fig. 1. Generation and Characterization of Embryonic Stem Cell Lines from NOD Blastocysts. (A) From blastocysts to ESCs: (a) A blastocyst stage embryo from a NOD female at day-3.5 p.c. was plated on MEFs in a 96-well plate; (b) the embryo started hatching from the zona pellucida on the day after plating and (c) the whole hatched embryo attached to MEFs at day 3; (d) ICM outgrowth was formed at day 5 of culture; (e) the outgrowth was dissociated into single cells using trypsin at this stage and the first passage ESC colonies were expanded on new MEFs, and (f) split when 80% confluent to form the second passage colonies. (B) Phase and corresponding immunofluorescence staining images of pluripotency markers (NANOG and OCT4) in the two male ESC lines continuously cultured in medium supplemented with BIO for 14 and 16 passages for KOBIO-3 and KOBIO-10, respectively. (C) Phase and corresponding immunofluorescence staining images of pluripotency markers (NANOG and OCT4) in the two male ESC lines cultured in medium with BIO for first 3 passages and then without BIO for 11 and 13 passages for ESC lines KOBIO-3 and KOBIO-10, respectively. (D) RT-PCR analysis of *Oct4*, *Nanog* and *Rex1* in male NOD ESC lines. (E) Karyotyping of male ESC lines. (F) H&E staining of teratoma showing tissues from the three germ layers: (a) keratinized-epithelium (asterisk, ectoderm), (b) secreting-gland epithelium (arrow, endoderm) and neuro-rosette (asterisk, ectoderm), (c) cartilage (arrowhead, mesoderm), (d) smooth muscle (arrowhead, mesoderm) and tubule inner epithelium (endoderm).