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SHORT REPORT

CD71^{high} population represents primitive erythroblasts derived from mouse embryonic stem cells

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Abstract The CD71/Ter119 combination has been widely used to reflect dynamic maturation of erythrocytes in vivo. However, because CD71 is expressed on all proliferating cells, it is unclear whether it can be utilized as an erythrocyte-specific marker during differentiation of embryonic stem cells (ESCs). In this study, we revealed that a population expressing high level of CD71 (CD71^{high}) during mouse ESC differentiation represented an in vitro counterpart of yolk sac-derived primitive erythroblasts (EryPs) isolated at 8.5 days post coitum. In addition, these CD71^{high} cells went through "maturational globin switching" and enucleated during terminal differentiation in vitro that were similar to the yolk sac-derived EryPs in vivo. We further demonstrated that the formation of CD71^{high} population was regulated differentially by key factors including Scl, HoxB4, Eaf1, and Klf1. Taken together, our study provides a technical advance that allows efficient segregation of EryPs from differentiated ESCs in vitro for further understanding molecular regulation during primitive erythropoiesis.

Introduction

Mammalian hematopoiesis is a dynamic process that is regulated spatially and temporarily through embryonic development (Baron and Fraser, 2005; Godin and Cumano, 2002; Palis et al., 1995). In mouse embryos, the initial wave of primitive hematopoiesis occurs around 7.5 days post coitum (dpc) at extraembryonic yolk sac (YS) and is characterized by large nucleated erythrocytes, megakaryocytes, and macrophages (Baron and Fraser, 2005; Godin and Cumano, 2002; Palis et al., 1995). The successive wave of definitive hematopoiesis can be detected in extraembryonic YS as well and in the intra-embryonic aorta-gonad-mesonephros region at later time point around 9.5 dpc (Baron and Fraser, 2005; Godin and Cumano, 2002 Palis et al., 1995). The second wave of hematopoiesis produces multi-lineage blood progenitors including definitive erythroid and lymphomyeloid lineages that are relocated to fetal liver (FL) before birth and finally to postnatal bone marrow (BM) (Baron and Fraser, 2005; Godin and Cumano, 2002 Palis et al., 1995).

The primitive erythroblasts (EryPs) generated from the initial hematopoiesis are the first blood lineage that differentiates and circulates within the embryos (Palis, 2008). They play critical roles in the transport of oxygen and carbon dioxide

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throughout embryos, as well as in facilitating vascular remodeling. EryPs distinguish from definitive erythroblasts (EryDs) in their larger size, unique globin expression, and oxygen carrying capacity (Baron et al., 2012; Fraser, 2013; Palis et al., 2010). In addition, although both lineages share a number of common regulators such as GATA1 and Klf1, recent transcriptome analyses have demonstrated that several transcriptional regulators are expressed at much higher levels in EryPs than those in EryDs, including *Pbx1*, *Foxh1*, *Arid3a*, *Pdlim7*, and *Cited2* (Isern et al., 2011; Kingsley et al., 2013). By contrast, a number of genes (including *Sox6*, *Nr3c1*, *Cebpa*, *Myb*, and *Irf9*) are highly enriched only in the EryDs from the FL and BM (Isern et al., 2011; Kingsley et al., 2013). The most dramatic change in terminal maturation of erythrocytes is enucleation. It was previously believed that circulating EryPs retained their nuclei, whereas EryDs enucleated in the FL or in adult BM, before entering the bloodstream. However, recent studies on mouse embryos and human embryonic stem cells (ESCs) have revealed that EryPs also enucleate and undergo progressive maturation similarly to EryDs (Fraser et al., 2007; Kingsley et al., 2006; Kingsley et al., 2004; Malik et al., 2013; McGrath et al., 2008).

Although EryPs are essential for embryonic survival, studies on their development and maturation in vivo are limited by the restricted numbers of EryPs and the technical difficulties to access them at early embryonic stages. ESCs have the potential to proliferate unlimitedly and to differentiate into various types of hematopoietic cells in vitro (Kyba et al., 2002; Wang et al., 2005). It has been demonstrated that Flk1+ hemangioblasts with bi-potential to differentiate into endothelial and hematopoietic lineages are precursors of EryPs (Choi et al., 1998). The CD71/Ter119 combination has been widely used, and its differential expression pattern correlates well with the maturation stages during development of both EryPs and EryDs in vivo (Fraser et al., 2007; Zhang et al., 2003). Although Ter119 is associated with glycophorin A and specifically marks maturing erythrocytes, CD71 is a transferrin receptor that is widely expressed on proliferating cells (Levy et al., 1999). It was reported that almost all embryoid bodies (EBs) differentiated from mouse ESCs (mESCs) expressed CD71 (Carotta et al., 2004), therefore it is unclear whether CD71 can be used as an erythrocyte-specific marker during ESC differentiation.

In this study, we found that CD71 was expressed on all ESC-derived cells at different levels, nevertheless, EryPs were highly enriched in the CD71^{high} subpopulation. In addition, these CD71^{high} cells went through "maturational globin switching" and enucleated when cultured in vitro. We further demonstrated that several core hematopoietic transcription factors differentially regulated the development of CD71^{high} population from mESCs. We here proposed a technical advance to segregate EryPs from surrounding cells for further analysis of the molecular mechanisms regulating the formation and maturation of erythrocytes.

Methods and materials

Cell culture and ESC differentiation

Mouse ESCs were cultured on irradiated MEFs as previously described (Wang et al., 2013). For EB formation, dissociated ESCs were plated to allow MEFs to adhere in IMDM/15% FBS (Invitrogen) with 0.2 mg/ml iron-saturated transferrin (Calbiochem), 4.5 mM α-monothiolglycerol (MTG, Sigma), and 50 μg/ml ascorbic acid (Sigma). Non-adherent cells were seeded in hanging drops at 500 cells per 15 μl drop. EBs were collected at day 2 into slowly rotating petri dishes. To induce specific gene expression, doxycycline (Dox) was added to the medium starting from day 2 at 1 μg/ml. Cells were harvested by collagenase treatment for analyses. MEL were cultured in RPMI-1640/10% FBS (Invitrogen). Detailed protocols to establish gene expressing or knockdown constructs and Dox-inducible ESC lines were described in Supplementary information.

In vitro erythroid differentiation and mouse tissue collection

Sorted cells were seeded onto fibronectin-coated plates (BD Biosciences) in IMDM/15% FBS, 1% detoxified BSA (Invitrogen), 0.2 mg/ml transferrin, 10 μg/ml human insulin (Roche), 0.1 mM MTG, and 2 U/ml human EPO (R&D). For OP9 co-culture, 10^4 OP9 cells per well were seeded onto 12-well plates overnight, followed by 5×10^5 sorted cells the next day in IMDM/20 ng/ml SCF/50 U/ml IL-3 (Peprotech), 10 ng/ml human insulin, 1 mg/ml transferrin, and 10 U/ml human EPO. For macrophages co-culture, adherent cells from BM in IMDM/10% FBS and M-CSF for 5 days, then co-cultured with sorted CD71^{high} cells in IMDM/15% knockout serum (Invitrogen), 10% protein free hybridoma II medium (Invitrogen), 0.15 mM MTG, and 2 U/mL erythropoietin for another 4–6 days. C57BL/6 mice were used for tissue collection and the age of embryos was defined as 0.5 dpc at noon on the day of vaginal plug observation. All animal experimental procedures were conducted in accordance with the local Animal Welfare Act and Public Health Service Policy.

Methylcellulose colony formation

1×10^4 sorted cells were mixed with 1.5 ml of MethoCult M3434 (Stem cell Technologies) following the manufacture's instruction. EryP and myeloid-macrophage colonies were counted respectively at day 5 and day 10 post plating.

Flow cytometry analysis and cell sorting

All antibodies used in this study were purchased as fluorochrome-coupled forms from BD Biosciences. Flow cytometry was performed with FACSCalibur (BD Biosciences) or with FACSAria II (BD Biosciences). DNA staining of live cells was performed with Vybrant DyeCycle™ Ruby (Invitrogen) according to the manufacture's protocol.

RT-PCR and quantitative PCR

Total RNAs were extracted with Trizol (Invitrogen) and cDNAs were synthesized using a PrimeScript® RT reagent Kit (TaKaRa Biotechnology Co., Ltd.). Real-time PCR was performed on a Stratagene MX3000P instrument and analyzed as described previously (Wang et al., 2013). Primers are listed in Supplementary Table S1.

Statistical analysis

All real-time PCR data were presented as the average of 4–6 replicates ± one s.e.m. from 2–3 independent experiments. For all other experiments, one representative experiment with biological duplicates was shown and results were reproducible for more than three times. p values were calculated using GraphPrism software for comparisons, *: $p < 0.05$; **: $p < 0.01$.

Results

A distinctive CD71^{high} population exists during differentiation of mESCs

To investigate if CD71 can be utilized as a marker for erythroblasts derived from mESCs, we monitored the percentage of CD71+ cells upon mESC differentiation by flow cytometry. CD71 appeared to be expressed at all EB cells differentiated from mESCs (Fig. 1A), consistent with its function as a proliferation marker as previously reported (Carotta et al., 2004). In addition, muscle cells were largely negative for CD71 (the few positive cells likely from contamination of blood cells in muscles), while distinctive CD71− and CD71+ populations were seen in BM as well (Fig. 1A). This suggests that the positivity of CD71 on all EB cells was not due to artificial draft caused by non-specific staining. More interestingly, we discovered that a CD71^{high} population existed along EB differentiation and peaked around day 5 (Fig. 1B). Only a small fraction of CD31+ or Flk1+ cells, were co-stained with CD71 around day 4 of EB differentiation (Fig. 1C), indicating that the segregation of CD71^{high} population from Flk1+ or CD31+ endothelial cells has already occurred at that time. At day 4 or day 6, we observed that a subpopulation of CD71^{high} cells was also positive for CD41 or c-Kit, markers of known primitive hematopoietic progenitors, but these cells were clearly CD45- and Ter119- (Fig. 1C). At day 8 of EB differentiation, double positive cells for CD71 and Ter119 emerged, indicating that some CD71^{high} cells matured into Ter119+ erythrocytes (Fig. 1C). These data distinguished the CD71^{high} population from CD31+ or Flk1+ endothelial cells and from CD45+ lymphoid–myeloid precursors as well, and thus proved the existence of CD71^{high} cells as potential erythroid progenitors.

CD71^{high} marks the primitive erythroblasts from mESCs

CD71 is expressed at high levels on several types of cells including erythroblasts and neurons (Levy et al., 1999). To define the property of CD71^{high} population, we sorted CD71^{low} (low level of CD71), CD71^{med} (mediate level of CD71), and CD71^{high} cells from day 5 EBs by flow cytometry (Supplementary Fig. S1A) and compared the expression levels of genes that were specific for erythrocyte development, megakaryopoiesis, and neuronal development. Compared to CD71^{low} and CD71^{med} cells, CD71^{high} cells expressed markedly higher levels of *Klf1* (the key factor in erythroid formation) and *βH1* (the embryonic hemoglobin) (Fig. 2A). In contrast, transcript levels of genes for endothelial growth (*Flk1* and *Tie2*), megakaryopoiesis (*Erg* and *Fli1*), and neuronal development (*Ttn* and *Lmx1b*) were significantly lower (Fig. 2A). To further determine if CD71^{high} cells represent EryPs, genes to be differentially expressed in EryPs and EryDs were examined. Notably, we found that genes reported to express at higher levels in EryP (such as *Pbx1*, *Foxh1*, and *Cited2*) were enriched in CD71^{high} populations and YS at 8.5 dpc (Fig. 2B). In contrast, genes defining EryD identity (such as *Sox6* and *Cepba*) were expressed at a much lower level in CD71^{high} cells than in CD71$^{low/med}$ fractions and FL at 14.5 dpc (Fig. 2B). In addition, similarly to EryPs from YS at 8.5 dpc, *βH1* embryonic hemoglobin was the dominant type of globin in CD71^{high} cells, in contrast to the EryDs from FL and adult BM, which expressed dramatically high levels of adult type globin *β1* (Fig. 2C). Taken together, these data suggest that the CD71^{high} population from differentiated mESCs represents an in vitro counterpart of EryPs from YS at 8.5 dpc in vivo.

To further characterize the CD71^{high} population, sorted cells were subjected to Wright–Giemsa staining. We found that the CD71^{high} population consisted of cells with uniform size and single nuclei, while CD71$^{low/med}$ fraction appeared to be more heterogeneous (Fig. 2D). We next determined their hematopoietic potential by colony forming assay. In this assay, about 800 red colonies per 10,000 CD71^{high} cells appeared around day 5 post plating and displayed the same morphology as erythroblast colonies from YS at 8.5 dpc. By contrast, few erythroblast colonies were detected in CD71$^{low/med}$ cells (Figs. 2E & F). We only found 16 colonies that mainly consisted of granulocytes and macrophages from CD71^{high} cells and 3 in CD71$^{low/med}$ groups. Cells collected from these EryP colonies displayed similar size and morphology as YS-derived erythroid progenitors, in that their nuclei became smaller after maturing in MethoCult for 5 days (Fig. 2G). Taken together, our results suggest that the erythropoietic potential was largely restricted to the CD71^{high} population from day 5 EBs.

Hemoglobin switching and enucleation of CD71^{high} cells during maturation

As development proceeds, EryPs in vivo continue to mature in the bloodstream and "maturational globin switching" occurs from dominant *βH1* at 8.5 dpc to comparable levels of *βH1* and *εy* globins (Kingsley et al., 2006). We examined if CD71^{high} cells could mature in vitro. After growing in vitro for two days, CD71$^{low/med}$ cells attached to the bottom of plates, whereas fractioned CD71^{high} cells existed in suspension (Fig. 3A). More importantly, although levels of all globins increased, the *εy* globin expression upregulated more significantly and comparable levels of *βH1* and *εy* transcripts were observed in CD71^{high} cells after culturing for two days in vitro (Fig. 3B). This phenomenon mimics the maturation process of EryPs from YS in vivo (Kingsley et al., 2006). In addition, when cultured for 3 days with cytokines including EPO on OP9, a stromal cell line that supports hematopoietic induction from ESCs (Nakano et al., 1994; Wang et al., 2005), the pellet from CD71^{high} cells turned red, an indicator of erythrocyte maturation (Fig. 3C). Similarly, "maturational globin switching" was detected as well in

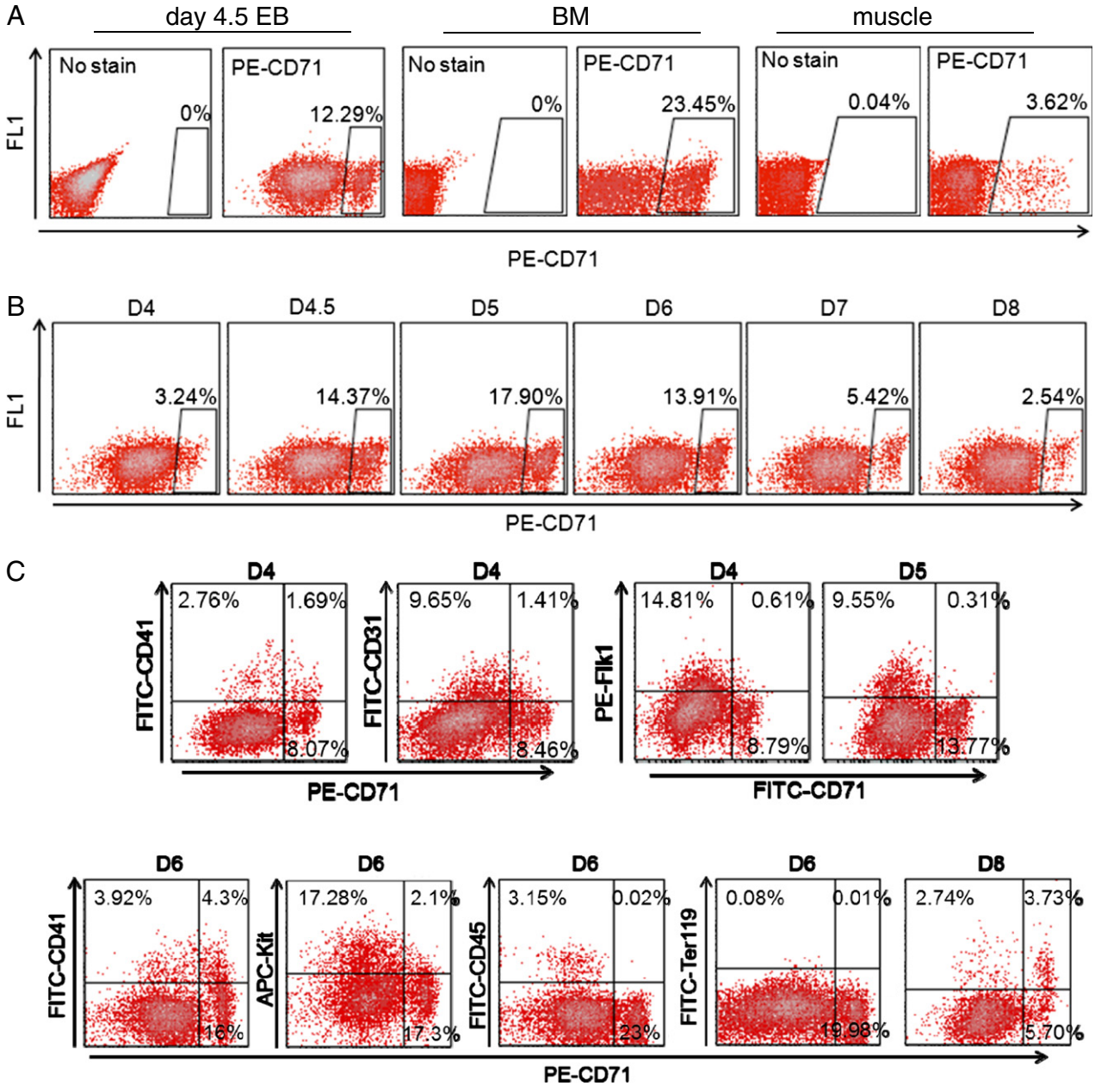

Figure 1 CD71high population exists along EB differentiation. (A–B) Flow cytometry analyses of CD71 on EBs, BM, and muscle cells (A) or on EB cells at various time points during ESC differentiation (B). (C) Staining of CD71 with markers for endothelial cells (CD31 and Flk1), hematopoietic progenitors (CD41), myeloid–lymphoid lineages (CD45), or for maturing erythrocytes (Ter119).

CD71high cells upon co-culturing with OP9 cells (Supplementary Fig. S1F).

However, despite that globin switching occurred during in vitro maturation of CD71high cells, no Ter119+ or enucleated cells were observed during these processes (data not shown), indicating an incomplete differentiation of CD71high cells under this condition. Since Ter119+ cells were detected in day 8 EBs, we further explored whether these CD71high cells could mature into Ter119+ or enucleated cells in the niche of EB microenvironment. In this assay, day 5 wildtype EBs were dissociated and re-aggregated with sorted GFP+CD71high or GFP+CD71low/med cells and cultured for four more days. We observed a higher percentage of Ter119+ cells in GFP+ (9.62%) than GFP− (3.08%) group derived from the re-aggregated EBs with GFP+CD71high cells, while comparable formation of Ter119+ population was seen in those from EBs mixed with GFP+CD71low/med cells (Fig. 3D). In addition, enucleated erythrocytes were detected in sorted GFP+Ter119+ cells as shown by Wright-Giemsa staining (Fig. 3D). Vybrant DyeCycle™ Ruby is a dye that only stains DNA of live cells, and thus terminal differentiated erythrocytes without nuclei will not be stained. We observed that in Ter119+ fraction about 2–3% of cells that were stained negative for this dye, 5 times more than those detected in Ter119− cells, indicating an existence of enucleated Ter119+ erythrocytes. We further adapted a protocol developed by McGrath et al. to induce enucleation of EryPs by co-culturing

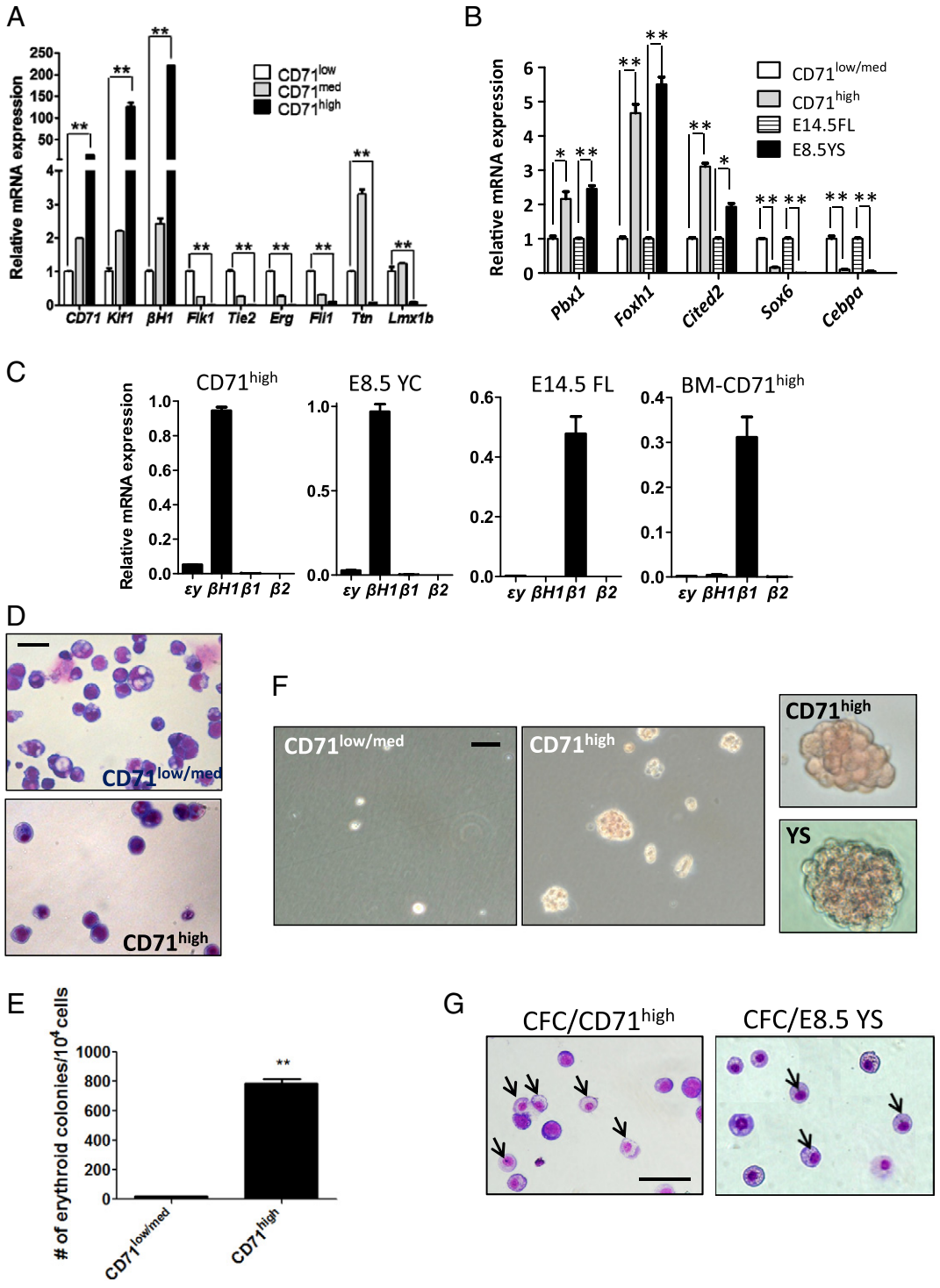

Figure 2 CD71^{high} population represents EryPs differentiated from mESCs. (A–C) Real-time RT-PCR on SSEA1-sorted day 5 EB cells, YS at 8.5 dpc, FL at 14.5 dpc, and CD71+ EryDs from adult BM. (D) Morphology of sorted CD71$^{low/med}$ and CD71^{high} cells. Scale bars: 50 μm. (E–F) Colony forming assay on CD71$^{low/med}$, CD71^{high} cells from day 5 EBs, and YS isolated at 8.5 dpc. Scale bars: 100 μm. Panels on the right show images with higher magnification. (G) Morphology of cells collected from erythroblast colonies of YS and CD71^{high} 5 days post plating. Scale bars: 50 μm.

CD71^{high} cells with macrophages (McGrath et al., 2008). We found that the enucleated erythrocytes were increased to 10% from the Ter119+ population when CD71^{high} cells were co-cultured with BM-derived macrophages (Fig. 3E). Therefore, our data supported the notion that putative EryPs from ESC-derived CD71^{high} cells were able to undergo "maturational globin switching" and enucleation in vitro.

Formation of CD71^{high} EryPs is regulated by transcription factors

The specification and terminal maturation of erythroblasts are regulated by various signals and transcription factors (Isern et al., 2011). To explore if CD71^{high} population can be used as a model to evaluate the function of transcription factors in primitive hematopoiesis, we established several doxycycline (Dox) inducible ESC lines and explored the effects of key transcription factors in the formation of CD71^{high} EryPs during ESC differentiation (Supplemental Figs. S2A–B). It was previously reported that the lineages

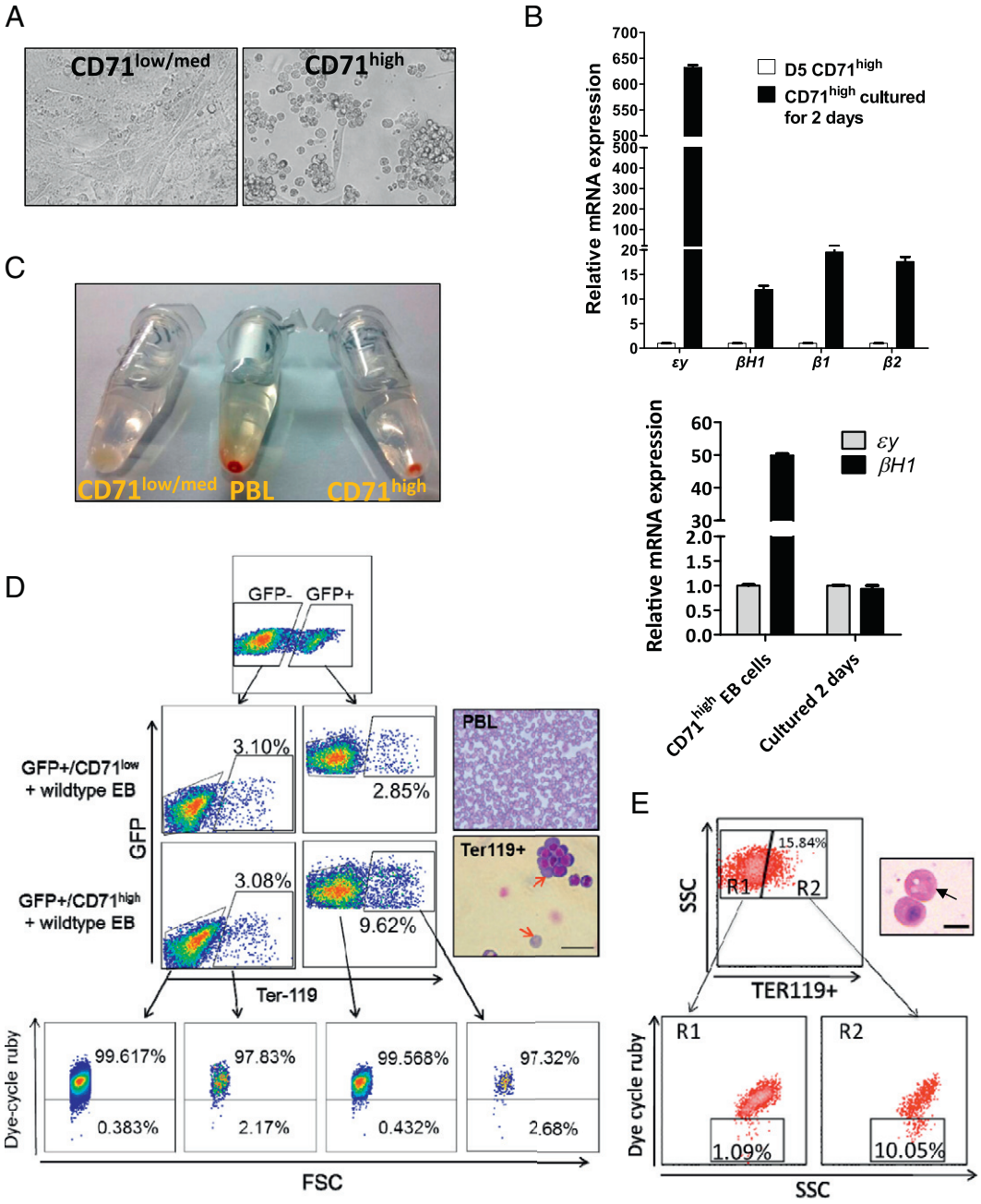

Figure 3 In vitro maturation of CD71^{high} cells. (A) Morphology of CD71$^{low/med}$ and CD71^{high} cells from day 5 EBs growing in vitro for two days. (B) Real-time RT-PCR analyses of hemoglobin expression on day 5 EB-derived CD71^{high} cells and CD71^{high} cells growing in vitro for two days. The relative expression of *βH1* to *εy*-globin in CD71^{high} population and CD71^{high} cells growing in vitro for two days was shown in lower panel. (C) Pellets from CD71$^{low/med}$, peripheral blood (PBL), and CD71^{high} cells growing on OP9 for three days. (D) Flow cytometry analyses of GFP+CD71$^{low/med}$ or GFP+CD71^{high} cells which were re-aggregated with wildtype EBs and cultured for 4 more days. Wright–Giemsa staining of PBL and sorted Ter119+GFP+ cells was shown on the right. Scale bars: 50 μm. (E) CD71^{high} cells co-cultured with BM-derived macrophages analyzed for Ter119 positivity and enucleation. Wright–Giemsa staining of sorted Ter119+ cells was shown on the right. Scale bars: 20 μm.

of erythrocytes and megakaryocytes in primitive hematopoiesis were generated from bipotential precursors (Klimchenko et al., 2009; Tober et al., 2007). We found that ectopic expression of Fli1, Erg, and HoxB4 from day 2 to day 5 of EB differentiation inhibited the formation of CD71^{high} EryPs (Fig. 4A, Supplementary Fig. S2F). By contrast, acting as a key regulator in erythropoiesis, Klf1 overexpression significantly promoted the development of

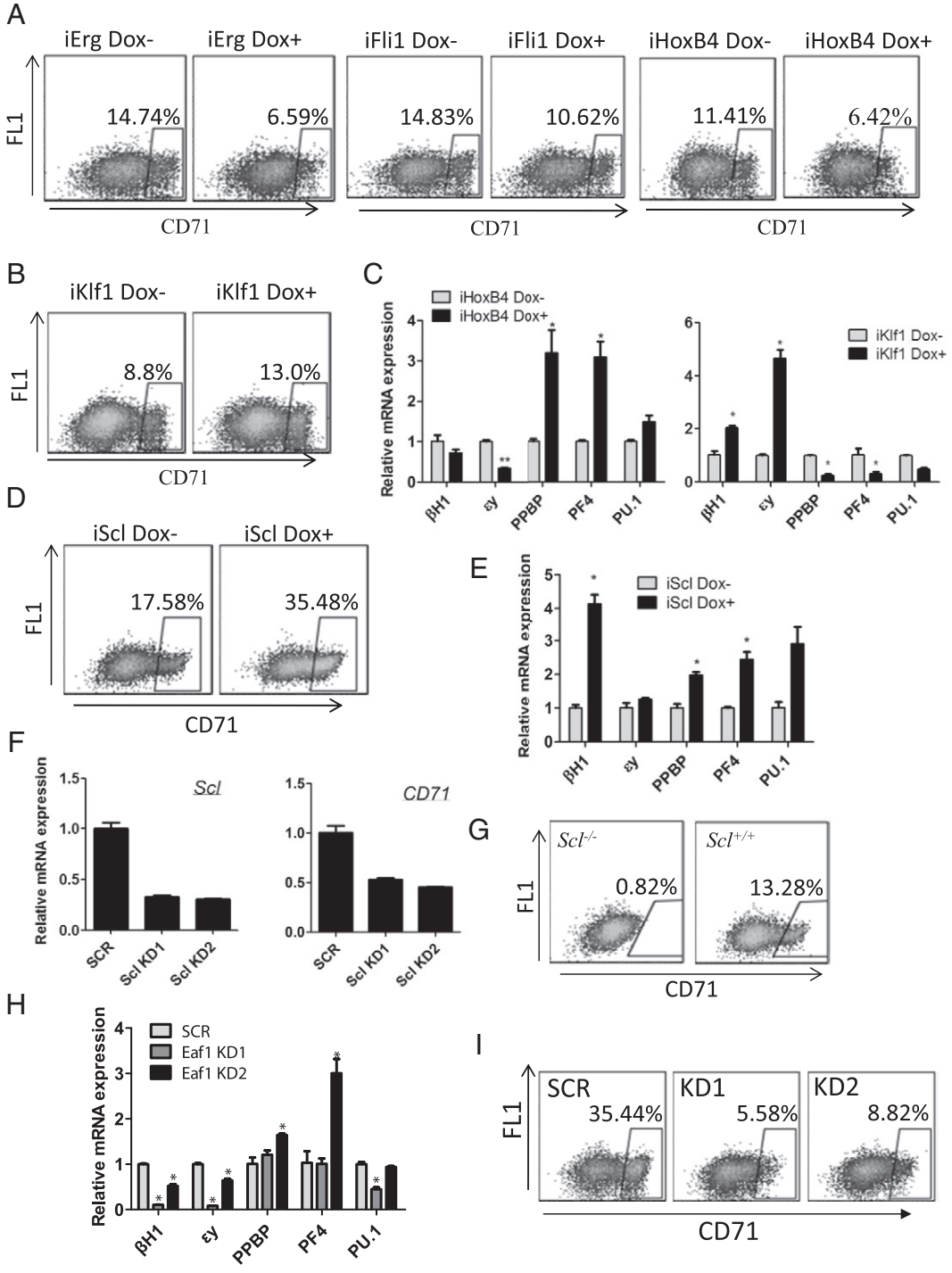

Figure 4 Formation of CD71^{high} population is regulated differentially by key transcription factors in hematopoiesis. (A–B) Flow cytometry analyses of CD71 on day 5 EBs differentiated from Dox-inducible ESCs in the presence (Dox+) or absence of doxycycline (Dox−). (C) Transcript levels of genes in hematopoiesis were measured by real-time RT-PCR on day 5 EBs from doxycycline inducible ESCs. (D–E) Flow cytometry analyses of CD71 (D) and real-time RT-PCR (E) on day 5 EBs differentiated from doxycycline inducible Scl ESCs. (F) Expression of *Scl* and *CD71* in MEL upon *Scl* knockdown by two different shRNAs. (G) Flow cytometry analyses of CD71 on wildtype and Scl$^{-/-}$ EBs at day 5 post differentiation. (H–I) real-time RT-PCR (H) and flow cytometry analyses of CD71 (I) on day 5 EBs differentiated from ESCs with scrambled shRNA (SCR) or shRNA (KD1 & KD2) against *Eaf1*.

CD71^{high} cells from differentiated EBs (Fig. 4B, Supplementary Fig. S2F). Consistent with these findings, the transcript levels of *βH1* and *εy* globins were downregulated in HoxB4 overexpressing cells but upregulated in Klf1-induced day 5 EBs (Fig. 4C). On the contrary, levels of genes specific for megakaryopoiesis such as PF4 and PPBP were increased upon HoxB4 induction, but reduced when Klf1 was ectopically expressed (Fig. 4C). These data thus supported the notion that primitive megakaryocytes and erythrocytes come from the same precursor and one may be upregulated at the expense of another lineage.

Scl plays an important role in erythroid development and also acts as a master regulator for hematopoiesis (Aplan et al., 1992; Porcher et al., 1996). When induced by Dox, Scl significantly upregulated the formation of CD71^{high} EryPs at different time points along EB differentiation and promoted the expression of genes in erythroid formation, megakaryopoiesis, and myeloid development (e.g. PU.1) (Figs. 4D, E, & Supplementary Fig. S2C). Upon Scl knockdown in MEL, an erythroleukemic cell line, CD71 expression was significantly downregulated (Fig. 4F). More importantly, although low to mediate level of CD71 expression was still observed from *Scl*$^{-/-}$ EBs, the CD71^{high} population disappeared in *Scl* null cells (Fig. 4G). These data thus provide direct evidence that Scl regulates primitive erythropoiesis.

We further investigate whether newly identified genes in EryPs play a role in formation of CD71^{high} cells. Although no obvious changes in percentage of CD71^{high} cells were observed upon induced expression of Foxh1 or Cited2 by Dox (Supplementary Figs. S2E–F), depletion of Eaf1 (a gene that played a role in zebrafish hematopoiesis, Hu et al., 2014) by RNAi significantly inhibited the formation of CD71^{high} population and down-regulated expression of *βH1* and *εy* globins (Figs. 4H–I, Supplementary Fig. S2G), suggesting that Eaf1 is required for derivation of EryPs from mESCs.

Discussion

In vitro differentiation of ESCs closely mimics early embryonic development and can produce large quantity of hematopoietic cells. However, segregation of the EryPs from surrounding cells differentiated from mESCs has not been reported. Here, we demonstrated that CD71^{high} could be utilized as a marker to purify EryPs from differentiated ESCs. Although overlapped with markers for hematopoietic progenitors, CD41 and c-kit, the CD71^{high} population was exclusive for myeloid-lymphoid cells which were positive for CD45. In addition, CD71^{high} cells sorted from day 5 EBs appeared to be homogenous, highly enriched for erythroblasts that gave rise about 800 red colonies per 10,000 cells. These data clearly demonstrate that EryPs in the CD71^{high} population are the dominant blood lineage. As previously reported, EryPs differ significantly from EryDs in size, globin expression, and transcriptional regulation (Baron et al., 2012; Fraser, 2013; Palis et al., 2010). The molecular mechanisms underlying these differences are however only partially described because significant amounts of EryPs are required for such mechanistic investigation. Efficient production and segregation of CD71^{high} EryPs from differentiated ESCs will therefore allow us to explore these fundamental questions during erythropoiesis.

Similarly to EryPs from YS around 8.5 dpc, *βH1* was the predominant type of embryonic goblin in CD71^{high} cells. These putative CD71^{high} EryPs were able to go through "maturational globin switching" and exhibited similar expression pattern with YS-derived EryPs. More importantly, we further demonstrated that these CD71^{high} cells matured into Ter119+ enucleated red blood cells when co-culturing with macrophages in vitro. These data thus validate our claim that CD71^{high} population represents an in vitro counterpart of YS-derived EryPs that can mature and enucleate. Notably, enucleation of erythrocytes in vitro has been a challenge in the field of hematopoietic research. It will be of great interest to explore the specific contribution of macrophages may have during this process in the future study.

According to current model, bi-potential megakaryocyte-erythroid precursors (MEPs) exist during primitive hematopoiesis (Klimchenko et al., 2009; Tober et al., 2007). However, it remains unclear how the fate specification of EryPs and megakaryocytes is regulated. We found that Scl promoted the formation of all blood lineages from mESCs as a master regulator of hematopoiesis. In contrast, megakaryopoietic differentiation depends more stringently on Ets family members, such as Erg and Fli1. At the expense of megakaryocytic development, induction of Klf1 favored the commitment of erythrocytes by upregulating CD71^{high} population and transcript levels of *βH1* and *εy* globins. Similarly, Eaf1 depletion blocked the development of erythrocytes as demonstrated by reduced percentage of CD71^{high} cells, thus indicating a critical requirement of Eaf1 in the formation of EryPs. Therefore, our approach to utilize CD71^{high} EryPs from ESCs coupled with genetic modification may represent a valuable model to examine cell-intrinsic regulation of primitive erythropoiesis during early embryonic development.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scr.2014.11.002.

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