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Letter to the Editor

SCF-mediated γ-globin gene expression in adult human erythroid cells is associated with KLF1, BCL11A and SOX6 down-regulation

To the Editor,

In humans, switching from fetal to adult hemoglobin occurs during the perinatal period and requires developmental stage-specific changes in chromatin remodeling complexes and transcription factors to progressively lead to β-globin gene activation and repression of γ-globin gene expression. Variable levels of HbF persist into adulthood. This variability depends on many genetic loci: three major loci – Xmn1-HBG2 single nucleotide polymorphism, *HBS1L-MYB* intergenic region on chromosome 6q and a region of 14 kb in intron 2 of the *BCL11A* gene – have been identified as the main regulators of HbF level in humans [1]. The principle that elevated HbF ameliorates the severity of the β-hemoglobin disorders has been the driving force behind efforts to stimulate fetal hemoglobin production. Several pharmacologic agents (hypomethylating agents, histone deacetylase inhibitors, short-chain fatty acids) have been shown to reactivate and augment HbF expression. In addition to chemical inducers, HbF synthesis may be stimulated by hematopoietic growth factors, such as stem cell factor (SCF) [2]. SCF receptor and SCF play a central role in hematopoiesis and, particularly, in the control of erythropoiesis [2–4]. Although much is known regarding trans-acting regulators and cis-acting elements within the core promoter and distal enhancer of γ-globin, the transcription factors through which SCF stimulates HbF synthesis in adult erythroid cells are largely unknown. To analyze the transcription factors through which SCF may stimulate HbF synthesis in human erythroid cells we employed erythroid unilineage cultures (E), derived from peripheral blood (PB) $CD34^+$ hematopoietic progenitor cells (HPCs) obtained from 6 independent volunteers, using experimental conditions previously reported [2]. In these serum-free liquid suspension cultures, purified HPCs are induced to selective E growth by very low dosages of IL-3, GM-CSF and saturating erythropoietin (Epo) level. These cultures were supplemented or not with 100 ng/ml SCF. Cell growth is associated with a gradual decrease of $CD34^+$ marker, followed by a progressively increasing expression of specific erythroid markers, such as glycophorin A (data not shown). Consistently, cell morphological analysis showed a gradual wave of maturation along the erythroid lineage up to terminal mature erythroid cells (Fig. 1A). In line with previously published results [3,4], SCF sharply stimulated erythroid cell proliferation, as indicated by a 2- to 3-log rise of cell output over control values, while delaying by about one week terminal differentiation/maturation (Fig. 1A). PB E cultures, grown in the absence of exogenous SCF, showed low γ-globin mRNA levels; low γ-globin-protein levels were observed both by Western Blot analysis and HPLC assay (Fig. 1B). Particularly, in these cultures, $\gamma / \beta + \gamma$ globin ratio is consistently less than 3%. In PB E cultures supplemented with SCF, a remarkable increase of HbF synthesis was observed, as consistently supported by mRNA and γ-globin protein evaluations (Fig. 1B). In these cultures, the $\gamma / \beta + \gamma$ globin ratio is around 24% (8 fold increase with respect to the control). It has been demonstrated that HbF induced by SCF is mediated mainly by ERK1/2 MAPK pathway. ERK1/2 phosphorylation resulted in γ-globin reactivation via down-regulation of the transcriptional repressor COUP-TFII in human erythroid cells [5]. First, we performed western blot analyses using anti-ERK antibodies for detection of total and phosphorylated ERK protein. Cells cultured in the absence of SCF showed no ERK activation; conversely, in the presence of SCF, ERK was phosphorylated as indicated by immunoblot analysis (Fig. 1C). Then, we analyzed whether SCF may modify the expression profile of other transcription factors involved in γ-globin gene expression such as GATA-1, GATA-2 and NF-YA [6]. The expression analysis of these transcription factors during differentiation/maturation of erythroid control cells showed a trend in line with previously published results [6]. None of these proteins were modulated by SCF treatment (Fig. 1C). A clear correlation of elevated HbF with low level of c-myb, and vice-versa, was evidenced by c-myb-overexpressing erythroid cell and genome-wide association studies, showing that polymorphisms in the myb locus are important mediators of variations in HbF levels [1,7]. During erythropoiesis, c-Myb expression is highest in CFU-Es and early erythroblasts, whereas its down-regulation appears to be required for terminal erythroid differentiation. Consistently, in our unilineage erythroid cultures c-myb protein sharply decreases during the differentiation process. Treatment with SCF did not substantially modify this trend: the decrease occurs later consistent with the delayed terminal differentiation/maturation induced by SCF (Fig. 1C). Recent genetic studies focused on natural variation of HbF expression level in human population established BCL11A as a new regulator of both developmental control of hemoglobin switching and silencing of γ-globin expression in adults [1,8]. Lentiviral vector mediated knockdown of BCL11A in erythroid cells was associated with increased levels of γ-globin and HbF production without affecting erythroid differentiation. The exact mechanism by which BCL11A silences γ-globin expression still remains unclear. A recent study suggests that this may be mediated through both interactions with transcription factors, such as SOX6, that binds chromatin at the proximal γ-globin promoters and through long-range interactions with a variety of regions throughout the γ-globin gene cluster [9]. We then analyzed SCF effect on BCL11A and SOX6 expression pattern. In primary adult human erythroid cells, BCL11A is predominantly expressed as two major extra-long (XL) and long (L) isoforms, whereas embryonic erythroleukemia cell line (K562), fetal liver and primitive erythroblasts express shorter variants, associated with high γ-globin synthesis. In line with published results [8], in our adult PB derived unilineage E cultures the shorter BCL11A isoforms were undetectable, whereas BCL11A XL and L isoforms were highly and uniformly expressed during all stages of erythroid differentiation, and consistent with low level expression of γ-globin chains. At late days of culture when erythroid maturation and hemoglobinization occur, BCL11A protein levels were markedly down-regulated in SCF treated erythroid cultures compared to control cells, consistent to the increased expression of γ-globin mRNA and protein. A similar behavior was shown by SOX6 transcription factor. We next evaluated the expression pattern of KLF1 in response to SCF stimulation. KLF1 is an essential

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transcription factor for proper function and development of the erythroid lineage and plays a critical role in regulating the developmental switch between fetal and adult hemoglobin expressions [10]. In fact, knockdown of KLF1 in human erythroid progenitors reduces BCL11A levels and increases γ-globin gene expression [10]. Expression of KLF1 was reduced in SCF-treated cultures as compared to untreated unilineage erythroid cultures (Fig. 1C). In conclusion, our results indicate that SCF-induced HbF reactivation is mediated by the coordinated down-modulation of three repressors of γ-globin transcription, BCL11A, SOX6 and KLF1. These observations further support a potential role of SCF as a re-activator of HbF synthesis under erythropoietic stress conditions and, hence, as a potential therapeutic agent in β-hemoglobinopathies.

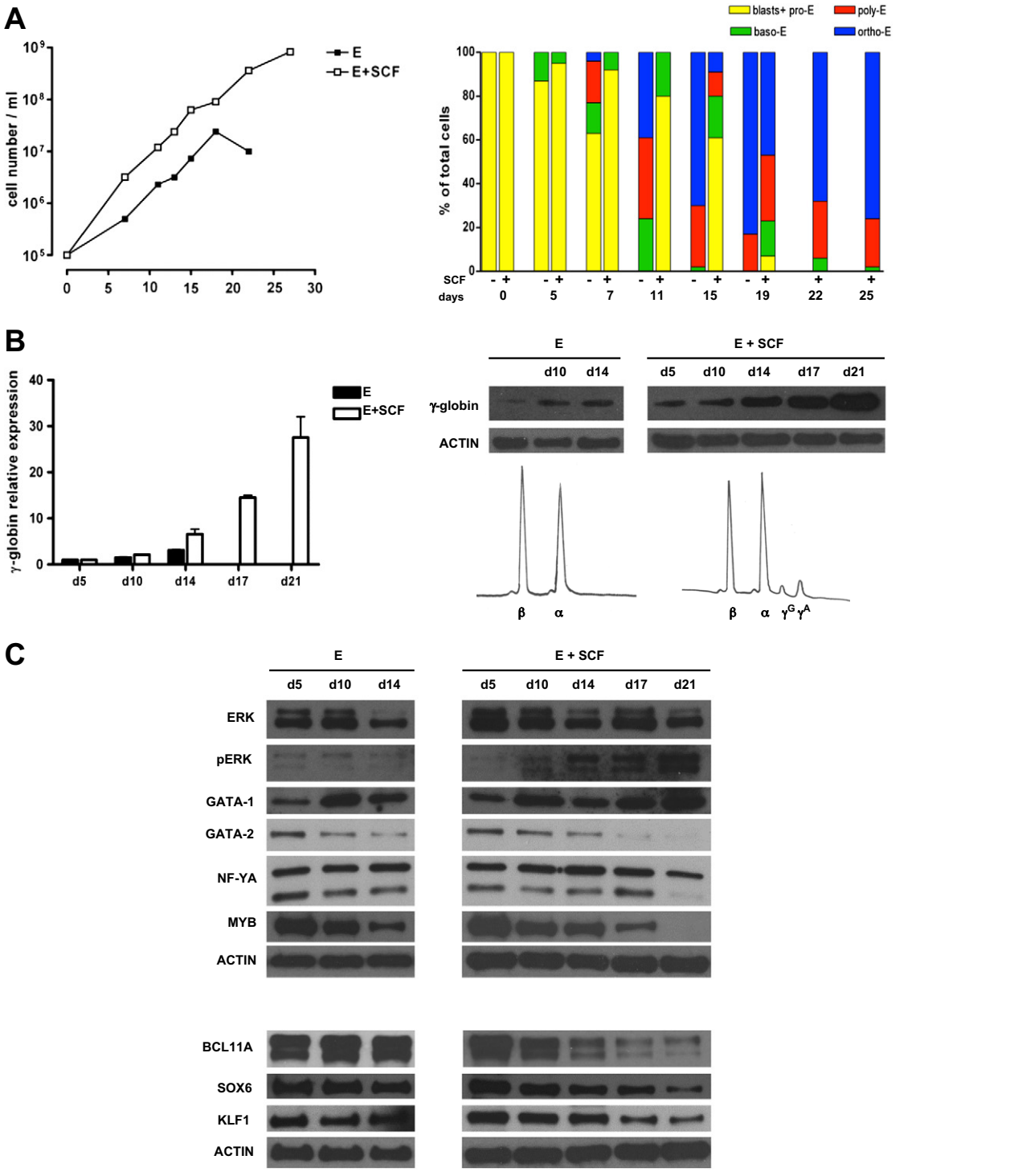

Fig. 1. A) Growth and differentiation of PB HPC erythroid (E) unilineage cultures supplemented or not with SCF. Percentage of blasts, differentiating proerythroblasts, basophilic (baso-E), polychromatophilic (poly-E) and mature orthocromatic (ortho-E) erythroblasts with respect to total cells is indicated. B) Expression of γ-globin analyzed by Real-time PCR and Western Blot in E cultures treated or not with SCF. γ-globin mRNA were detected by Syber Green system and normalized against human GAPDH transcript levels. Error bars represents standard deviation and indicate the average values from three independent experiments. Representative Western Blot and HPLC scan of globin chains synthesis in mature erythroblasts (day 14 of culture) from six independent donors are shown. C) Analysis of transcription factors by Western blotting. Total cell extracts were prepared from differentiating erythroid cells treated or not with SCF. Actin was used as loading control. Representative experiments are presented.

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