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ETS transcription factors in hematopoietic stem cell development

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ABSTRACT

Hematopoietic stem cells (HSCs) are essential for the maintenance of the hematopoietic system. However, these cells cannot be maintained or created in vitro, and very little is known about their generation during embryogenesis. Many transcription factors and signaling pathways play essential roles at various stages of HSC development. Members of the ETS ('E twenty-six') family of transcription factors are recognized as key regulators within the gene regulatory networks governing hematopoiesis, including the ontogeny of HSCs. Remarkably, although all ETS transcription factors bind the same DNA consensus sequence and overlapping tissue expression is observed, individual ETS transcription factors play unique roles in the development of HSCs. Also, these transcription factors are recurrently used throughout development and their functions are context-dependent, increasing the challenge of studying their mechanism of action. Critically, ETS factors also play roles under pathological conditions, such as leukemia and, therefore, deciphering their mechanism of action will not only enhance our knowledge of normal hematopoiesis, but also inform protocols for their creation in vitro from pluripotent stem cells and the design of new therapeutic approaches for the treatment of malignant blood cell diseases. In this review, we summarize the key findings on the roles of ETS transcription factors in HSC development and discuss novel mechanisms by which they could control hematopoiesis.

Introduction

Hematopoiesis, including the generation of hematopoietic stem cells (HSCs) during embryogenesis, is a complex process in which multiple regulatory pathways converge to orchestrate tissue formation, cell movement and cell fate decisions including lineage specification and differentiation [1]. Defining the molecular mechanisms underlying these processes is essential to understanding how their development takes place and is clinically relevant for regenerative medicine. Transcription factors execute the gene regulatory networks controlling the multiple processes involved in hematopoiesis, integrating the multiple endogenous and environmental clues which influence cell behavior. Within the hematopoietic system, a number of transcription factors have been shown to be essential, including several members of the ETS transcription factor family.

ETS transcription factors comprise a large evolutionarily conserved gene family characterized by sequence homology within their DNA binding ETS domain, which forms a winged helix-turn-helix structural motif that binds to the consensus sequence, GGAA/T, the ETS binding site found in target genes [2]. ETS transcription factors are involved in the regulation of a variety of biological processes and are present in all of the metazoan genomes analyzed to date [3]. The number of ETS transcription factors has increased in vertebrates compared to invertebrate metazoans: while in *Drosophila* and *Caenorhabditis elegans*, 8 and 10 genes, respectively, are present, in vertebrates 26 or more members of this family have been found [2–5]. ETS gene radiation in vertebrates might be related to the development of a sophisticated hematopoietic system and the emergence of a closed circulatory system. Although hematopoiesis takes place in *Drosophila* and the orthologs of well characterized vertebrate blood transcription factors (Runx, Gata, Fog) regulate this process, no members of the ETS family have been shown to be involved [6]. *Drosophila* hematopoiesis produces phagocytes which comprise the innate immune system and are analogs of vertebrate myeloid cells [7]. In vertebrates, the development of myeloid cells is controlled by one of the most versatile master regulators of hematopoietic fate decisions, the ETS transcription factor PU.1 [8–10]. Strikingly, PU.1 and other closely related ETS transcription factors, SpiB and SpiC, have only been identified in vertebrates [11,12] where they control not only the development of the innate but also of the adaptive immune system [8]. Thus, it appears that new ETS transcription factors emerged and co-evolved with the vertebrate adaptive immune system. In vertebrates, a large number of ETS transcription factors are expressed and play essential roles in hematopoietic cells and the vasculature, with the gene regulatory networks controlling the development of these tissues containing ETS transcription factors at their core. For example, an ETS-FoxC interaction controls expression in endothelial cells [13] while an ETS-Gata interaction is at the core of hematopoietic expression [14,15].

Abbreviations: HSC, hematopoietic stem cell; VEGF, vascular endothelial growth factor.

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The ETS family comprises activators and repressors as well as proteins with both activation and repression activities [16]. In hematopoietic and endothelial cells, a number of ETS transcription factors are co-expressed at any single time, suggesting that a degree of functional redundancy may exist or that they act in a combinatorial manner to control development. Nevertheless, even if all ETS transcription factors bind the same consensus sequence and several members of the family may be present in a particular tissue at a specific time in development, a wealth of evidence indicates that individual ETS transcription factors play distinct, non-overlapping functions. Here, we discuss the key roles that ETS transcription factors play in hematopoiesis, in particular in the ontogeny of the HSC.

The role of Etv2, Etv6 and Fli1 in the specification of the hematopoietic lineage from nascent mesoderm

In mammalian and avian embryos, hematopoietic specification initiates when nascent mesodermal cells of the primitive streak are specified as cardiovascular progenitors, some of these progenitors migrate to the yolk sac to become hemangioblasts, the presumptive common progenitor cells for blood and endothelium. Expression of Flk1/VegfR2 by Brachyury-expressing cells is thought to mark the emergence of hemangioblasts [17] and, therefore, it has been considered that Flk1 signaling initiates the gene regulatory network controlling hemangioblast development. In agreement, Flk1 deficiency in the mouse results in the absence of blood and endothelial cell development [18]. In the zebrafish, however, Flk1 deficiency is less significant for hemangioblast specification [19–21], but the *cloche* mutation impairs hemangioblast development [22,23]. Nevertheless, the identity of the gene(s) mutated in *cloche* has not been confirmed and therefore the precise role of this factor(s) has not been characterized in other models.

Although in zebrafish and *Xenopus* blood cell development is not completely dependent, Etv2/Er71/Etsrp is absolutely required for blood and endothelial cell development in the mouse [24]. Moreover, initial analysis of Etv2-deficient mouse embryos showed that Flk1 expression is dependent on Etv2, therefore suggesting that Etv2 sits at the top of the gene regulatory network controlling the emergence of blood and endothelial cell progenitors [24]. In support of this, Etv2 has been shown to bind Flk1's promoter in mouse cells [24] and directly activate its expression in *Xenopus* embryos [25]. In addition, Flk1 expression is absent in Etv2-deficient zebrafish embryos [5,26,27] and exogenous Etv2 can induce Flk1 expression in both fish and *Xenopus*, even in *cloche* mutants [25,27]. However, more recently, it has been demonstrated that in ES cells Etv2 is dispensable for the generation of $Flk1^+$ mesoderm; in Etv2-deficient ES cells, $Flk1^+$ cells are generated but their development is arrested [28]. Presumably, this occurs before hemogenic endothelium is specified and, as a consequence, blood cells are not generated [28,29]. Furthermore, vascular endothelial growth factor alpha (VEGFA) rapidly induces high levels of Etv2 expression in $Flk1^+$ cells, strongly suggesting that Etv2 is downstream of Flk1 [28]. To clarify the genetic hierarchy between Flk1 and Etv2, a transgenic Etv2-EYFP reporter mouse has shown that Etv2 expression is restricted to a subset of $Flk1^+$ cells during primitive streak stages [30]. Furthermore, the number of Flk1-expressing cells in Etv2-deficient embryos was only modestly reduced, while Etv2-EYFP expression was almost undetectable in Flk1-deficient embryos [30]. Additionally, it was demonstrated that the Etv2 promoter is activated by VEGFA-Flk1 signaling, strongly indicating that, in the earliest hematoendothelial progenitors emerging from the primitive streak, Flk1 is required for Etv2 expression [30]. This evidence indicates that Flk1 and Etv2 can activate each other bidirectionally in a context-dependent manner and that a feasible scenario would be that Flk1 is required for the initial expression of Etv2 but that, after hematoendothelial progenitors have been specified, Flk1 may require an Etv2 positive feedback for its expression in developing blood vessels.

All the observations presented above relate to the development of embryonic hemangioblasts which give rise to transient yolk sac embryonic hematopoiesis. Elucidation of the epistatic relationship between Flk1 and Etv2, at the time HSCs emerge from the hemogenic endothelium associated with the ventral wall of the dorsal aorta, has not been possible due to the complete absence of the dorsal aorta itself in both Flk1- and Etv2-deficient embryos in all models where it has been examined [12,18,25,27,31]. HSC emergence is intimately linked to the development of the dorsal aorta and hence understanding how the earliest progenitors to the dorsal aorta are programmed could reveal the earliest events in HSC programming. In the mouse, lineage labeling has demonstrated that all endothelial cells of the dorsal aorta, as well as all blood cells in the marrow, derive from lateral plate mesoderm [32–34]. Nevertheless, the exact location of the dorsal aorta/HSC progenitors is not known, nor is it known if they derive from a population of adult hemangioblasts. In zebrafish embryos, the dorsal aorta derives from hemangioblasts localized in the lateral plate but they are intermingled with embryonic hemangioblasts giving rise to primitive red cells [35] and therefore the programming of the adult as opposed to embryonic hemangioblasts cannot be analyzed easily. As in the mouse, in *Xenopus* embryos the dorsal aorta and adult hematopoiesis derive from lateral plate mesoderm [36–38]. The dorsal lateral plate mesoderm of *Xenopus* embryos contains a population of adult hemangioblasts which, in contrast to the zebrafish, have a distinct origin and anatomical location to that of the embryonic hemangioblasts and therefore we have exploited these advantages to study the earliest programming of the HSC lineage [31,38–40]. We have established that adult hemangioblast programming requires synergism between a VEGFA-dependent and a VEGFA-independent pathway, controlled by the ETS transcription factors, Etv6/Tel1 and Fli1, respectively (Fig. 1; [31]). The first synergistic interaction between these pathways takes place when VEGFA produced in the somites, under the control of Etv6, activates $Flk1^+$ cells in the dorsal lateral plate itself where Fli1 together with Gata2 ensure the expression of Flk1 (Fig. 1A); as a result, an endogenous, cell autonomous Etv6-VEGFA circuit is established. Simultaneously, in a VEGFA-independent manner, Fli1 and Gata2 activate the transcription of Etv2 (Fig. 1B) which in its turn activates Lmo2 and maintains Gata2 expression to set the second synergistic interaction between the VEGFA-dependent and VEGFA-independent pathways to ultimately activate Scl (Fig. 1C; [31,41]). Thus, during adult hemangioblast specification, Flk1 and Etv2 transcriptional control is mutually independent. The adult hemangioblast gene regulatory network also shows that two ETS transcription factors are at the top of the hierarchy, Etv6 and Fli1. Evidence in the zebrafish also indicates that Fli1 does not lie downstream of Etv2 since Fli1-expressing cells in the posterior lateral plate mesoderm can be detected in Etv2-deficient embryos [27]. The relationship between Etv6 and Etv2 has not been investigated in the mouse or any other system. Nevertheless, the adult hemangioblast gene regulatory network shows that ETS transcription factors, despite sharing the same DNA binding consensus sequence, play essential and unique functions in the establishment of the HSC lineage.

Using the ES cell differentiation system, it was initially demonstrated that hemangioblasts and cardiac cells derive from Flk1-expressing progenitors [42]. More recently, it has been demonstrated in embryos that hemangioblasts and cardiac cells are intimately related [43–46]. In zebrafish embryos, embryonic hemangioblasts and cardiac cells derive from a common population of mesodermal progenitors; within this population, an Fgf signaling regime dictates cardiogenic vs hemangioblast fate, favoring cardiac differentiation [44]. Moreover, a cross antagonism between hemangioblast and cardiac transcription factors has been demonstrated, in other words, hemangioblast transcription factors repress the cardiac fate whereas cardiac transcription factors repress the hemangioblast fate [43–46]. Etv2 has emerged as a key regulator of hemangioblast vs cardiac fate determination. Unlike Fli1, Gata2 and Flk1, whose expression persists after hemangioblast specification, Etv2 expression in ES cells is transient, taking place only between days 1–4 of differentiation [24,29]. Flk1 negative mesoderm isolated at day 3.5 of

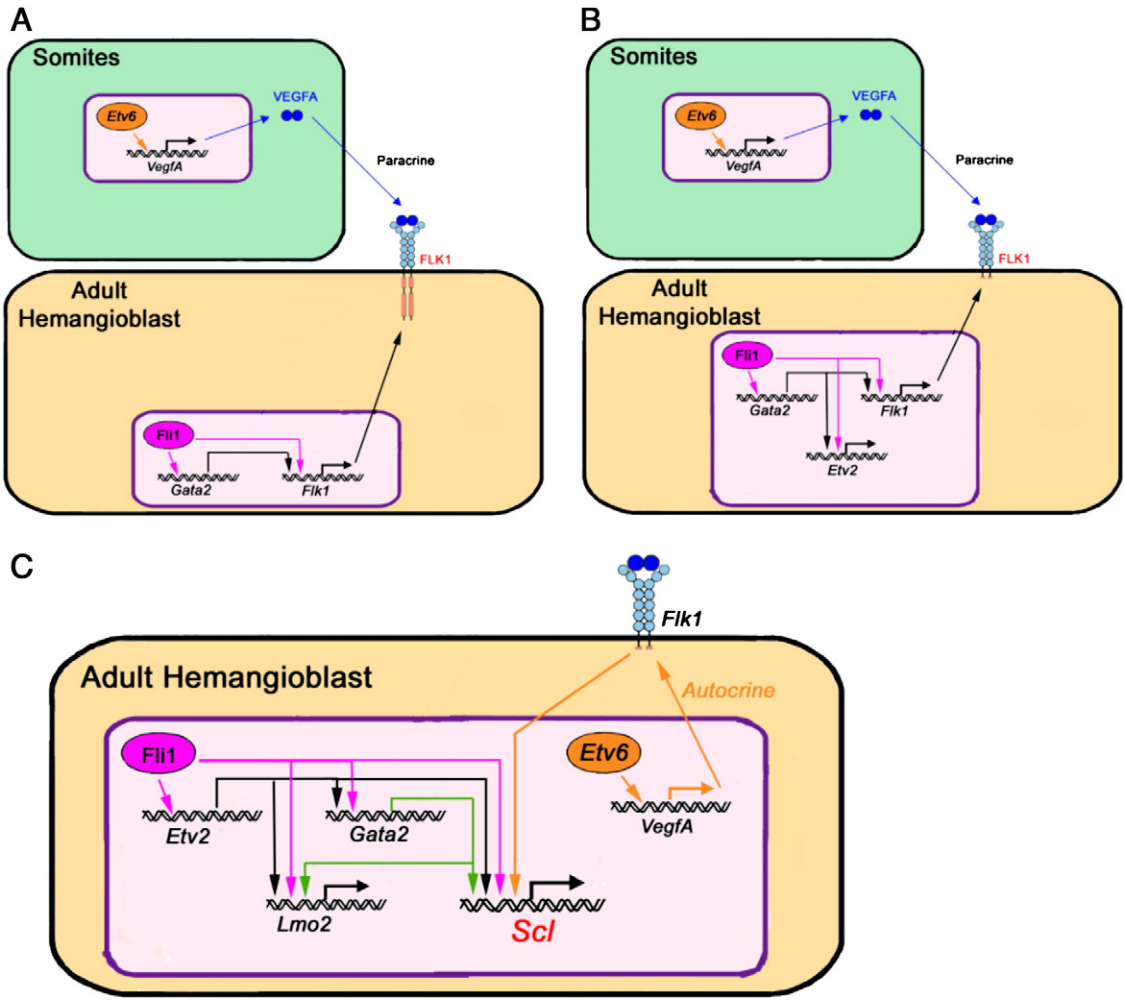

Fig. 1. ETS transcription factors are at the core of the gene regulatory network programming adult hemangioblasts in the lateral plate mesoderm. A. The adult hemangioblast program is initiated when VEGFA produced in the somites under the control of Etv6, activates its receptor, Flk1, in hemangioblasts precursors localized in the dorsal lateral plate mesoderm. Flk1 expression in hemangioblasts precursors is controlled by Fli1 and Gata2. B. Fli1 and Gata2 activate Etv2 expression in adult hemangioblasts precursors independently of VEGFA signaling. C. In a VEGFA-independent manner, Etv2 controls the expression of genes which synergize with cell autonomous VEGFA signaling to activate the expression of Scl, a gene essential for adult hemangioblast determination.

differentiation becomes Flk1$^+$ by day 4.25 [42], presumably in the absence of Etv2, further indicating that nascent cardiovascular progenitors do not require Etv2 to activate Flk1. This second wave of Flk1$^+$ cells gives rise to cardiac progenitors [42]. Furthermore, in the zebrafish, Etv2 is required to repress the cardiac fate in the anterior lateral plate mesoderm while favoring hematopoietic specification [47]. Thus, Etv2 modulates fate decisions in nascent cardiovascular progenitors where, on the one hand, it instructs a subpopulation of Flk1$^+$ cells, very likely through activation of Scl [45,48] or repression of Wnt signaling [49], to acquire the hematoendothelial fate while repressing their cardiac potential; and, on the other hand, Flk1$^+$ cells need to be released from Etv2 activity to express their cardiac potential (Fig. 2).

ETS transcription factors and vascular development

The emergence of HSCs is intimately associated with blood vessel endothelium. HSCs are generated from the endothelium of the major arteries of the developing embryo [50]. In particular, it has been shown that the first HSCs emerge in association with the ventral wall of the lumenised and fully functional dorsal aorta (see accompanying reviews in this issue). Therefore, understanding how the dorsal aorta is specified would inform on the mechanism by which HSCs are programmed. Deficiencies of most of the key hematopoietic transcription factors have an impact on hemangioblast specification which results in early embryo lethality in the mouse due to defects in primitive blood and blood vessel development, and as a consequence hematopoiesis in the dorsal aorta cannot be analyzed. In contrast, fish and frog embryos are less dependent on oxygen delivered by the circulation and embryos deficient in the same transcription factors survive long enough to analyze the emergence of HSCs in the dorsal aorta. Thus, the zebrafish and *Xenopus* models have contributed to our understanding of the development of the dorsal aorta/HSC precursors [51]. Using these models, it has been shown that the dorsal aorta and HSCs derive from hemangioblasts arising in the lateral plate mesoderm, then a subpopulation of these cells migrate to the midline where they coalesce to form the dorsal aorta. Subsequently, the dorsal aorta polarizes and hemogenic endothelium is specified on its ventral aspect where HSCs eventually emerge [51].

ETS transcription factors also feature prominently in the gene regulatory network controlling the development of the vascular system, including the lymphatic vessels [52]. Central to this gene regulatory network is a conserved Fox:ETS interaction [13,52] whereby, to drive endothelial expression, ETS factors function in cooperation with FoxC transcription factors via a composite element, the FOX:ETS motif [13,53]. FoxC2 and Etv2 can bind this motif simultaneously and activate all of the enhancers containing this motif [13]. Thus, coexpression of FoxC2 and Etv2 causes ectopic expression of endothelial genes in *Xenopus* embryos, while the combined knockdown of the orthologous genes in zebrafish embryos induces vascular defects [13]. Although

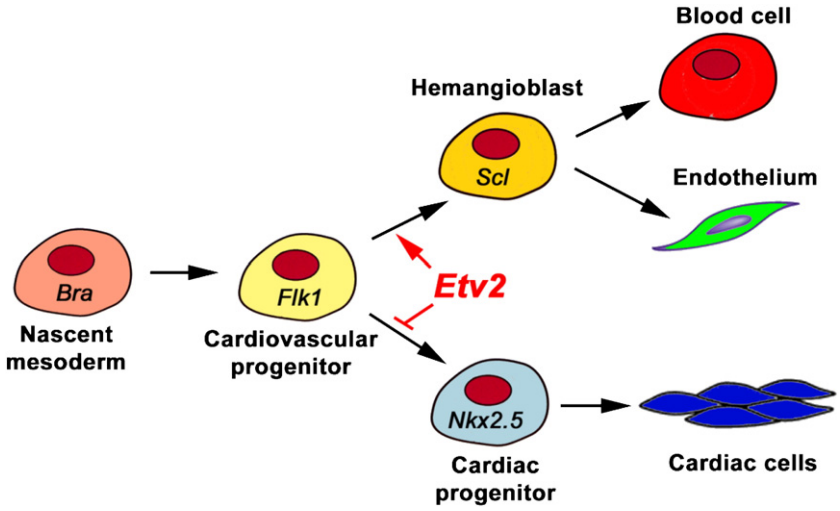

Fig. 2. Etv2 controls the hematopoietic potential of nascent cardiovascular progenitors. Expression of Etv2 in Flk1 + cardiovascular progenitors induces the hemangioblast program while repressing the cardiac fate. In the absence of Etv2 expression, Flk1 + cells differentiate into cardiac cells.

Etv2 and FoxC2 were originally reported to transactivate expression in endothelial cells, evidence suggests that other ETS and Fox transcription factors may utilize the Fox:ETS motif to control endothelial development and/or maintenance.

A number of ETS transcription factors are expressed in the developing and mature vasculature but only a few appear to impact vascular integrity. Apart from Etv2, the deficiency of single ETS transcription factors appears to have little impact on blood vessel development [54]. For example, Ets1, the founding member of the ETS family, is highly expressed in embryonic endothelial cells and can directly regulate many specific vascular genes [55,56]; nevertheless, in *ets1*-deficient zebrafish embryos, endothelial cells are still present and circulation is only lost after 24 h post-fertilization [57]. Fli1 is also highly expressed in the endothelial lineage and null mutations in the mouse results in lethality at E12.5 due to disrupted blood vessel integrity [58], but its deficiency in the zebrafish only displays a partial loss of trunk circulation and hemorrhage in the head [5]. Similarly, Elk3, a vascular specific ETS transcription factor, does not appear to be essential for vascular development [59]. This suggests that, within the vasculature, ETS transcription factors play redundant roles or that they are not involved in vascular integrity but rather other vascular functions. Indeed, Elk3 has been shown to be required for lymphatic vessel integrity [59] and, in Etv6-deficient embryos, vascular development takes place normally but arterial specification of the dorsal aorta fails and, subsequently, hemogenic endothelium/HSC programming fails [41].

The potential role of ETS factors in the endothelial to hematopoietic transition

The emergence of the first HSCs in the ventral wall of the dorsal aorta is thought to be by a novel process termed the endothelial to hematopoietic transition [60]. The runt domain transcription factor, Runx1, plays a key role in both the specification of hemogenic endothelium and endothelial to hematopoietic transition but the mechanism by which it controls these processes is currently unknown. Endothelial to hematopoietic transition superficially resembles the widely observed epithelial to mesenchymal transition and Runx factors have recently been shown to regulate epithelial to mesenchymal transition in malignant tissues [61]. Runx1, Runx2 and Runx3 are expressed in thyroid carcinomas, and analysis of Runx2 function demonstrated that expression of angiogenic factors, such as VEGFA and VEGFC, as well as epithelial to mesenchymal transition-related factors, such as Snail2, Snail3 and Twist1, are regulated by Runx2 [61]. Thus, during HSC emergence, it is possible that Runx1 controls endothelial to hematopoietic transition by regulating EMT-related factors.

A number of ETS transcription factors have been shown to play key roles in epithelial to mesenchymal transition in malignant tissues. On the one hand, Ets1 in renal epithelial cells [62], Elf5, Erf and Pdef in breast cancer cells [63–65], Ese3 in prostate cancer [66], and Etv5 in ovarian cancer cells [67] repress epithelial to mesenchymal transition. On the other hand, Erg in prostate cancer cells [68] and Etv4 in prostate, lung, ovarian and breast cancer cells [69,70], induce epithelial to mesenchymal transition. Epithelial to mesenchymal transition regulation in these malignant tissues generally involves the transcriptional regulation of key epithelial to mesenchymal transition transcription factors such as Snail1, Snail2, Twist1, Zeb1 and Zeb2; for example, Ets1 binds the promoter of Zeb1 and directly regulates its expression [71], while Etv4 regulates the expression of Twist1, Snail1, Zeb1 and Zeb2 [69]. Therefore, if epithelial to mesenchymal transition and endothelial to hematopoietic transition share a common molecular machinery, due to their expression in hemogenic endothelium, ETS transcription factors are very likely to be involved in the regulation of endothelial to hematopoietic transition during the emergence of HSC from hemogenic endothelium.

Runx1 and ETS transcription factors have been shown to interact at multiple levels. ETS transcription factors might be involved in Runx1 transcriptional regulation since a Runx1 enhancer, which drives its expression in emerging HSCs, contains ETS binding sites required for its activity and this enhancer can be bound by Fli1, Elf1 and Pu.1 [72]; reciprocally, Runx1 regulates the expression of hematopoietic ETS transcription factors [15,73]. Also, genome wide ChIPseq data indicate that Runx1 and ETS transcription factors co-regulate a large cohort of hematopoietic genes [15,74]. Importantly, Runx1 can physically bind ETS transcription factors [75–78]. It has been shown that the Runx1 physical interaction with ETS transcription factors is conserved in *Drosophila*, where Lozenge and Pointed, the orthologs of Runx1 and Ets1/Ets2 respectively, associate before DNA binding [78]. It was also shown that Runx1 interaction with ETS transcription factors is independent of the runt domain, it is the Lozenge ExonV in *Drosophila* and Runx1 ExonVI in mammals, which physically associates with the pointed domain of ETS transcription factors [78]. Therefore, any ETS transcription factor containing the pointed domain (Ets1, Ets2, Fli1, Erg, Gabpα, Elf3, Elf5, Ese3, Pdef, Etv6 and Etv7) is capable of physically associating with Runx1 and potentially co-regulating the endothelial to hematopoietic transition and other processes during HSC generation. Of the ETS transcription factors containing the pointed domain, Ets1, Erg, Elf5, Ese3 and Pdef have been shown to regulate epithelial to mesenchymal transition in cancer cells and therefore, if expressed in hemogenic endothelium, could drive

endothelial to hematopoietic transition together with Runx1. Thus, ETS transcription factors are very likely to be at the core of the endothelial to hematopoietic transition modulated by Runx1 and the generation of the first HSCs.

HSC specification and initiation

A number of transcription factors that play essential roles in HSC emergence are regulated by ETS transcription factors [79]. For example, Scl is important for the establishment of the hemogenic endothelium in mouse embryos [80] and, in zebrafish, Scl marks and is required for hemangioblast specification. Thus, its deficiency results in both hematopoietic and endothelial development defects, which lead to the absence of the dorsal aorta and definitive hematopoiesis [81,82]. Expression of *scl* in hemangioblasts and HSCs is regulated by an enhancer bound by a Gata2 and ETS transcription factor (Fli1, Elf1) protein complex [14]. Gata2, Fli1 and Scl have been shown to form a transcriptional complex on enhancers containing Gata, ETS and E-box binding motifs and formation of this protein complex drives expression of its targets specifically in hemangioblasts and HSCs [83]. Moreover, these transcription factors contain those DNA binding motifs in their own regulatory elements and co-regulate each other to form a self-regulating transcriptional circuit which maintains their expression in emerging HSCs [83]. As discussed above, Runx1 is essential in the endothelial to hematopoietic transition process generating HSCs in the dorsal aorta [84] and in vivo studies show that it can be directly regulated by GATA2 and ETS transcription factors. A Runx1 intronic enhancer containing Gata, ETS and E-box binding sites can recruit GATA2, Fli1, Elf1, Pu.1, and Scl in vitro, and in vivo experiments have shown that this enhancer is sufficient to drive expression in emerging HSCs, and therefore that it is responsible for Runx1 expression in these cells [72].

The ETS transcription factor, Fev (also known as Pet1), which was originally identified in serotonergic neurons in zebrafish and mammals [85–87], is also expressed in blood/endothelial cells in zebrafish [5]. We have demonstrated that Fev deficiency results in a reduction of Runx1 expression in the dorsal aorta and fewer T cells in the thymus, and that Fev regulates the number of Runx1 expressing cells by transcriptionally activating its target, Erk2, to enhance ERK signaling [88]. Thus, Fev is a novel ETS transcription factor involved in the genesis of HSCs.

ETS transcription factors, miRNAs and hematopoiesis

Non-coding RNAs, in particular microRNAs (miRNAs), play important roles in normal and malignant tissues and, therefore, their functions should be considered if we are to accurately understand developmental processes. miRNAs are a class of small non-coding RNAs that regulate gene expression by acting at the post-transcriptional level to repress multiple target genes via inhibition of translation or destabilization of mRNA [89]. miRNAs have been shown to be abundant during HSC differentiation in the bone marrow, and they appear to play key roles in HSC homeostasis, including HSC quiescence, self-renewal and ultimately fate decisions and differentiation (reviewed in [90]). Moreover, increasing evidence indicates that deregulation of miRNA expression is involved in the pathogenesis of leukemia since they can act as oncogenes and tumor suppressors [91,92]. Although the role of miRNAs in normal and pathological adult hematopoiesis is widely recognized, their role during developmental hematopoiesis is poorly understood.

Whereas little is known about the regulation of miRNAs generally, ETS transcription factors have a significant role in the regulation of hematopoietic and endothelial miRNAs. Primitive erythropoiesis in the yolk sac and differentiating embryonic stem cells initiate with the emergence of progenitors capable of giving rise to erythrocyte colony-forming cells. These progenitors are generated transiently and miR-126 has been demonstrated to stop the development of erythrocyte colony-forming cells [93]. Although some of the downstream targets of miR-126 in this process have been identified, nothing is known about its own regulation. However, in endothelial cells, miR-126 expression is regulated by Ets1 and Ets2 [94]. Likewise for zebrafish miR-142a-3p, which has been demonstrated to regulate vascular development and integrity, its targets in these particular processes have been identified but its regulation is unknown [95]. miR142 is of particular interest because it is one of the most abundantly expressed miRNAs in blood cells and it appears to be a blood-specific miRNA [96]. In agreement, zebrafish miR-142-3p influences the differentiation of primitive erythrocytes but its mechanism of action has not been defined [97]. Importantly, the promoter of miR-142 contains an essential ETS binding site which in primary bone marrow derived dendritic cells, as well as cultured macrophages, is bound and activated by PU.1 [98]; in this context, PU.1 synergizes with Runx1 and C/EBPβ to confer hematopoietic specific expression [98]. PU.1 has also been demonstrated to directly regulate at least 4 other miRNAs (miR-146a, miR-342, miR-338 and miR-155) that are required for macrophage differentiation [99]. This indicates that PU.1's function in myeloid differentiation is partly modulated by miRNAs and opens the possibility that PU.1's influence in acute myeloid leukemia (AML) may be modulated by miRNAs such as miR-142-3p [100]. In the same vein, it has recently been reported that PU.1, together with Fli1, directly activate miR-17-92 (also known as Fli3) in friend erythroleukemia and, in its turn, miR-17-92 regulates cell proliferation [101]. In the same system, Fli1 represses the expression of miR-17 and miR-20a, two miRNAs involved in cell proliferation arrest [101]. PU.1 has also been demonstrated to indirectly repress the expression of miR-17-92 during macrophage differentiation by inducing the expression of Egr2 which directly represses miR-17-92 [102]. Thus, ETS transcription factors are capable of controlling miRNA expression both positively and negatively to modulate normal hematopoiesis and malignant cell progression.

miRNAs have been shown to influence endothelial and hematopoietic differentiation in some cases by regulating ETS transcription factor expression. For example, in endothelial cells, both miR-199a-5p and miR-200b target Ets1 to block angiogenesis [103,104]. miR-155, a miRNA highly expressed in human cord blood $CD34^{+}$ hematopoietic progenitor cells but weakly expressed in megakaryocytes, blocks megakaryocyte differentiation when induced in HPCs while its down regulation enhances their differentiation [105]. Further analysis indicated that Ets1, a key factor in megakaryocyte differentiation, is targeted by miR-155 [105]. miRNAs also have the capacity to control ETS transcription factor expression in malignant blood cells. For instance, acute

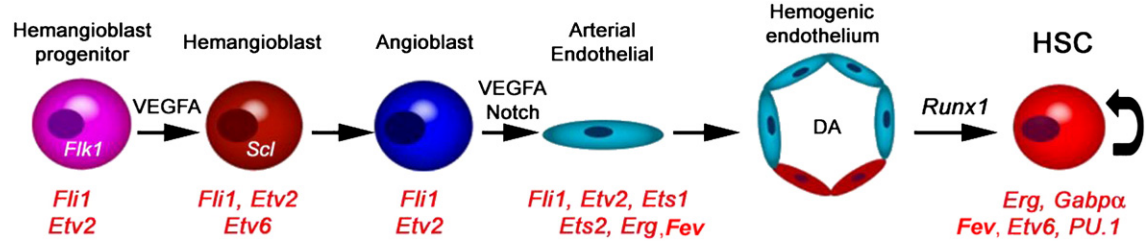

Fig. 3. ETS transcription factors essential for the programming of the hematopoietic stem cell lineage. Schematics showing the cellular hierarchy in the programming of HSCs in developing embryos. ETS transcription factors known to be critical for the development or maintenance of each cellular type are indicated.

myeloid leukemia patients with NPM1 mutations have higher levels of miR-196a and miR-196b that regulate Erg, an adverse prognostic factor in patients with acute myeloid leukemia and T-acute lymphoblastic leukemia [106]. Thus, the epistatic relationship between ETS transcription factors and miRNAs constitutes an integral part of normal and pathological gene regulatory networks, and research in this field may result in novel strategies to produce blood cells, including HSCs, in vitro as well as for designing new therapies to treat malignant blood diseases.

Conclusion and perspectives

The hematopoietic system serves as a paradigm of development and stem cell biology and exemplifies how transcription factors, signaling molecules and new regulators, such as miRNAs, integrate into gene regulatory networks controlling multiple aspects of development. It appears that ETS transcription factors are at the core of the gene regulatory networks controlling the key aspects of hematopoietic specification during embryogenesis as well as the maintenance and differentiation of HSCs (Fig. 3). The importance of this family of transcription factors is patent in their direct involvement in the pathology of blood diseases. Although all ETS transcription factors control gene expression by binding to the same consensus sequence, it is clear that they exhibit essential, non-overlapping functions and therefore deciphering how these unique functions are achieved is a major challenge for the understanding of normal and pathological hematopoiesis. Addressing how ETS transcription factors interact with other transcription factors, such as Runx1, signaling pathways and miRNAs is also a major challenge. In addition, evidence indicates that the regulation and function of single ETS transcription factors, such as PU.1, are context dependent and therefore that their functions must be studied in a tissue-specific manner. Thus, it is clear that a multidisciplinary approach is required to undertake these challenges. Undoubtedly, the study of ETS transcription factors will contribute to a better understanding of developmental hematopoiesis and therefore will help to instruct protocols for the differentiation of HSC towards particular blood lineages as well as the generation of HSCs in vitro and, ultimately, therapies for the treatment of malignant blood diseases and regenerative medicine.

Conflict of interest

The authors declare no competing financial interests.

Acknowledgments

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