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Tel1/ETV6 Specifies Blood Stem Cells through the Agency of VEGF Signaling

Aldo Ciau-Uitz,[1,2] Philip Pinheiro,[1,2] Rajeev Gupta,[1] Tariq Enver,[1] and Roger Patient[1,*]
[1]MRC Molecular Haematology Unit, Weatherall Institute of Molecular Medicine, University of Oxford, John Radcliffe Hospital, Headington, Oxford OX3 9DS, UK
[2]These are co-first authors
*Correspondence: roger.patient@imm.ox.ac.uk
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SUMMARY

The regulation of stem cell ontogeny is poorly understood. We show that the leukemia-associated Ets transcription factor, Tel1/ETV6, specifies the first hematopoietic stem cells (HSCs) in the dorsal aorta (DA). In contrast, Tel1/ETV6 has little effect on embryonic blood formation, further distinguishing the programming of the long- and short-term blood populations. Consistent with the notion of concordance of arterial and HSC programs, we show that Tel1/ETV6 is also required for the specification of the DA as an artery. We further show that Tel1/ETV6 acts by regulating the transcription of *VegfA* in both the lateral plate mesoderm and also in the somites. Exogenous VEGFA rescues *Tel1/ETV6* morphants, and depletion of VEGFA or its receptor, Flk1, largely phenocopies Tel1/ETV6 depletion. Few such links between intrinsic and extrinsic programming of stem cells have been reported previously. Our data place *Tel1/ETV6* at the apex of the genetic regulatory cascade leading to HSC production.

INTRODUCTION

Hematopoietic stem cells (HSCs) are first detected in the floor of the embryonic dorsal aorta (DA) in all vertebrates studied (reviewed by Dzierzak and Speck [2008]). A mechanistic understanding of their formation and maintenance in an undifferentiated state will facilitate not only better manipulation and/or generation of these cells for therapeutic use, but also a better understanding of the corruption of these properties in leukemia. Indeed, one approach to identifying the nuclear controls over HSC formation and behavior has been to analyze the phenotypic consequences of knocking out the functions of transcription factors (TFs) associated with leukemia (Pina and Enver, 2007). Thus, many of the leukemic TFs have been knocked out in mice, and have been shown to affect blood stem cell numbers in the adult bone marrow and also in several cases in the embryonic DA. However, it has not been possible in many cases to distinguish an effect on formation from an effect on maintenance of stem cells. In order to study this, we have exploited the technical advantages of the amphibian, *Xenopus laevis*, and have charted the cellular history of HSCs during development (Ciau-Uitz et al., 2000; Walmsley et al., 2002). We have shown that their development is separate from the primitive blood from the 32 cell stage of development, and that they pass through a hemangioblast-like intermediate formed in the dorsal lateral plate (DLP) mesoderm, which is characterized by the coexpression of blood- and endothelium-associated genes. A subset of this hemangioblast-like population switches off the blood gene expression and migrates to the midline, where they turn on the arterial program and form the DA (Ciau-Uitz et al., 2000). The DA becomes polarized with blood gene expression returning in the floor, but not the roof, reflecting the formation of hemogenic endothelium, and eventually HSCs emerge there. In this paper, we have used this system to explore the role played by the leukemic TF, Tel1/ETV6, in HSC formation.

The ETS TF, Tel1/ETV6, henceforth referred to as Tel1, was initially identified by virtue of its chromosomal rearrangement in human chronic myelomonocytic leukemia (Golub et al., 1994). *Tel1*$^{-/-}$ mouse embryos die at Embryonic Day (E) 10.5 to E11.5 due to impairment of blood vessel development, and *Tel1*$^{-/-}$ cells are unable to contribute to bone marrow hematopoiesis (Wang et al., 1997, 1998). In order to determine if the requirement was for the formation or the maintenance of HSCs, conditional knockouts in hematopoietic cells were engineered, and suggested that Tel1 is only required for the survival of HSCs in the bone marrow (Hock et al., 2004). These data, together with the chimera data (Wang et al., 1998), excluded a cell-autonomous role for Tel1 in HSC development, but a non-cell-autonomous role was not addressed in these experiments.

In this paper, we revisit the role of Tel1 in HSC development, and show that it is indeed required. Exploiting the knowledge of the cellular history and the technical advantages of the *Xenopus* system, we track the requirement back to the hemangioblast-like cells in the DLP mesoderm, and show that known targets of VEGF signaling are affected. We therefore tested whether VEGFA production was affected, and showed that it is. We also showed that VEGFA can rescue the Tel1 loss-of-function phenotype, that VEGFA signaling from both somite tissue and within the DLP itself are required for hematopoiesis, and that knockdown of *VegfA* or its receptor, *Flk1*, phenocopies much of the *Tel1* phenotype. We therefore conclude that Tel1 plays a critical role in HSC development, controlling VEGFA production in and around the precursors. These observations may have significance for the mechanism by which HSC numbers and leukemia are sustained.

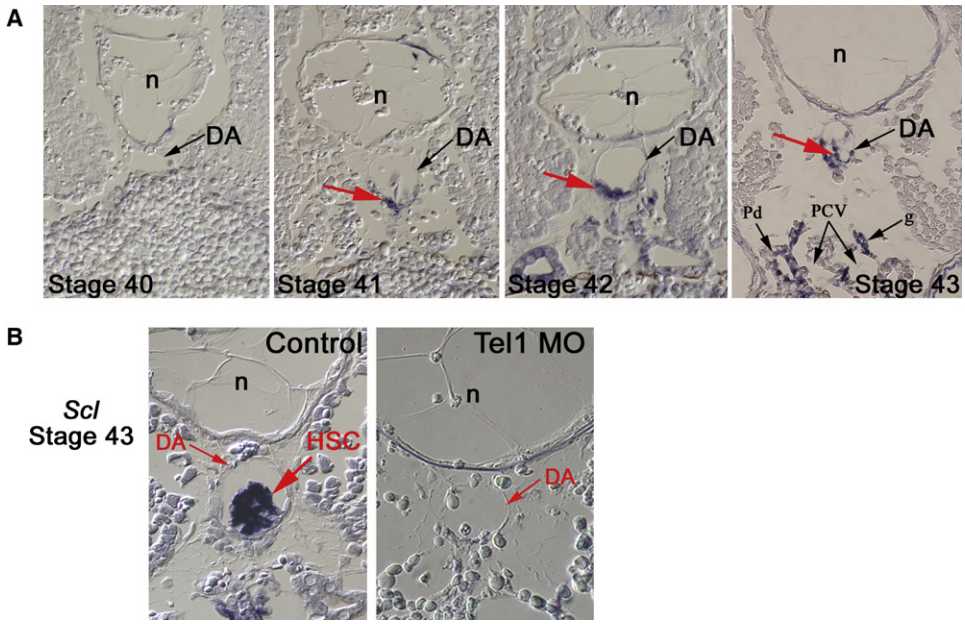

Figure 1. *Tel1* Is Expressed in the Embryonic DA and Is Required for the Emergence of HSCs

(A) In situ hybridization on transverse sections showing expression of *Tel1* in the ventral wall of the DA and emerging HSCs (red arrows).

(B) *Scl* staining on transverse sections shows that intra-aortic hematopoietic clusters containing HSCs do not emerge in *Tel1* morphants. All sections shown are at 40× magnification, with dorsal to the top. g, ganglia; n, notochord; PCV, posterior cardinal vein; Pd, pronephric duct. Stages of development are as indicated. See also Figures S1 and S2, and Table S1.

RESULTS

Tel1 Is Expressed in and Required for Emerging HSCs

Expression analysis by in situ hybridization of *Tel1* in *Xenopus* embryos showed that *Tel1* is expressed in the floor of the DA from stage 41 (Figure 1A, red arrows), when the HSCs are beginning to emerge (Ciau-Uitz et al., 2000). It is therefore in place to play a role in that emergence. We tested this by knocking down its activity with antisense morpholinos (MOs). The design and controls for the MOs are described in Supplemental Information (see Figure S1 available online). Significantly, HSC emergence was inhibited in these embryos: neither morphological clusters nor expression of hematopoietic markers, such as *Scl*, could be seen (Figure 1B). We therefore conclude that Tel1 is required for the formation of HSCs in developing embryos.

The hematopoietic program initiates in the floor of the DA, and the cells eventually round up before budding out starting around stage 42/43 (Ciau-Uitz et al., 2000). To begin to place *Tel1* in the hierarchy of gene expression leading to HSC formation, we monitored expression of *Runx1*, *SpiB*, *Scl*, *Lmo2*, and *Gfi1* in the DA of *Tel1* morphants at stage 39, nearly 1 day before HSC emergence (Figure 2A, red arrows). None of these HSC-associated genes were expressed. In contrast, expression in primitive erythroid cells was unaffected, confirmed by quantitative RT-PCR (qPCR) analysis of α-globin expression (Figure S1F), although more cells remained in the VBI due to a circulation defect (Figure 2A, red arrowheads). In addition, the arterial endothelial program, exemplified by *Notch4*, *Dll4*, and *EphrinB2*, which initiates along with *Gata2* when the precursors arrive at the midline 10 hr before *Runx1* expression commences, was also silenced (Figure 2B, red arrows). In contrast, expression in the DA of the pan-endothelial markers, *Erg1* and *Tie2*, was unaffected (Figure 2C; Figure S1G, red arrows), which, together with the later observation of a fully lumenized vessel (Figure 1B), confirms that endothelial specification, migration, coalescence, and lumen formation all took place apparently normally. We therefore conclude that Tel1 plays a very specific role in establishing the hematopoietic and arterial programs in the DA.

Tel1 Programs Adult Hemangioblasts

Despite the clear evidence that *Tel1* is required long before HSC emergence in the DA, we could find no evidence for its expression there at these times (Figure 1A and data not shown). We therefore reasoned that it must be expressed earlier. The DA and its derivatives, the HSCs, are derived from the DLP mesoderm in *Xenopus* embryos (Ciau-Uitz et al., 2000; Walmsley et al., 2002). We therefore carried out in situ hybridization on embryos at stages 24–28, when cells coexpressing blood and endothelial markers can be detected there, before migration to the midline. *Tel1* expression was clearly detected in these cells (Figure 3A, red arrows). We therefore monitored, in Tel1 morphants, the expression of some of the blood and endothelial genes normally expressed there. Of the three main blood genes expressed there, we found a clear diminution of *Scl* expression, but not *Gata2* or *Lmo2* (Figure 3B, red arrows). As controls, expression of these genes in the primitive erythroid population was maintained (Figure 3B, red arrowheads). For the endothelial genes tested, we found substantial reduction of *Flt1* and *Flt4* expression, whereas *Flk1* expression was not significantly affected (Figure 3C, red arrows). We therefore conclude that *Tel1* is indeed expressed in the precursors to the DA and