SOX7 expression is critically required in FLK1-expressing cells for vasculogenesis and angiogenesis during mouse embryonic development

Andrew J. Lilly a, Andrzej Mazan a, Daryl A. Scott b, Georges Lacaud a,c, Valerie Kouskoff a,c,⁎⁎

a Cancer Research UK Manchester Institute, The University of Manchester, Wilmslow road, M20 4BX, UK
b Department of Molecular and Human Genetics, Baylor College of Medicine, One Baylor Plaza, BCM227, Houston, TX 77030, USA
c Division of Developmental Biology and Medicine, The University of Manchester, Michael Smith Building, Oxford Road, Manchester M13 9PT, UK

A R T I C L E  I N F O

Article history:
Received 27 January 2017
Received in revised form 27 April 2017
Accepted 31 May 2017
Available online 31 May 2017

Keywords:
Vascular development
SOX7
Endothelium

A B S T R A C T

The transcriptional program that regulates the differentiation of endothelial precursor cells into a highly organized vascular network is still poorly understood. Here we explore the role of SOX7 during this process, performing a detailed analysis of the vascular defects resulting from either a complete deficiency in Sox7 expression or from the conditional deletion of Sox7 in FLK1-expressing cells. We analysed the consequence of Sox7 deficiency from E7.5 onward to determine from which stage of development the effect of Sox7 deficiency can be observed. We show that while Sox7 is expressed at the onset of endothelial specification from mesoderm, Sox7 deficiency does not impact the emergence of the first endothelial progenitors. However, by E8.5, clear signs of defective vascular development are already observed with the presence of highly unorganised endothelial cords rather than distinct paired dorsal aorta. By E10.5, both Sox7 complete knockout and FLK1-specific deletion of Sox7 lead to widespread vascular defects. In contrast, while SOX7 is expressed in the earliest specified blood progenitors, the VAV-specific deletion of Sox7 does not affect the hematopoietic system. Together, our data reveal the unique role of SOX7 in vasculogenesis and angiogenesis during embryonic development.

© 2017 The Authors. Published by Elsevier Ireland Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

1. Introduction

The development of the vascular system involves a complex array of processes necessary to regulate the dynamic nature of the emerging vascular network. During development, the first blood vessels form in the extra-embryonic yolk sac via vasculogenesis, which initiates following the formation of blood islands from mesodermal progenitors (Ferkowicz and Yoder, 2005). Cells on the inside of the blood islands differentiate into blood cells, whereas cells on the outside differentiate into endothelial precursor cells (EPCs), which migrate and associate to form a primitive vascular plexus (Herbert and Stainier, 2011). In the embryo proper, EPCs migrate to form endothelial chords that differentiate into the major arteries and veins (De Val and Black, 2009). The primitive extra and intra-embryonic vascular network subsequently undergoes angiogenesis involving the remodelling and expansion of blood vessels resulting in the formation of a hierarchically organized vascular network (Risau and Flamme, 1995). The transcriptional network regulating the identity and behaviour of EPCs involved in vascular development is extremely complex and remains poorly understood.

The Sox family of genes encodes a group of transcription factors that all share a high mobility group (HMG) DNA binding domain and recognise the AACAAT consensus sequence (Scheper et al., 2002). The SOX F subgroup contains SOX7, SOX17 and SOX18, and a growing body of evidence indicates that they have important roles in cardiovascular development (Francois et al., 2010; Lilly et al., 2017). However, SOX17 has pleiotropic functions and regulates a variety of processes including: definitive endoderm specification (Kanai-Azuma et al., 2002), fetal hematopoietic stem cell proliferation (Kim et al., 2007), oligodendrocyte development (Sohn et al., 2006) and arterial specification during cardiovascular development (Corada et al., 2013). The role of SOX18 appears to be more restricted with deficiency in this factor leading to specific defects in lymphangiogenesis (Francois et al., 2008). In contrast, the role and function of SOX7 is still poorly defined. SOX7 is expressed in primitive endoderm (Futaki et al., 2004; Murakami et al., 2004) and in endothelial cells at various stages of vascular development. These include the mesodermal masses that give rise to blood islands in gastrulating embryos (Gandillet et al., 2013), and the vascular endothelial cells of the dorsal aorta, intersomitic vessels and cardinal veins in more developed embryos (Hosking et al., 2009; Kim et al., 2016; Takash et al., 2001). Gross morphological examination of Sox7−/− mouse embryos suggests potential vascular defects (Wat et al., 2012); more recently, it was...
shown that the conditional deletion of Sox7 in Tie2 expressing endothelial cells results in branching and sprouting angiogenic defects at E10.5 (Kim et al., 2016). Despite these recent advances, a comprehensive analysis of the developing vascular network encompassing both vasculogenic and angiogenic processes in SOX7 deficient embryos has not yet been undertaken.

**Fig. 1.** SOX7 is expressed at the onset of endothelial differentiation from mesodermal precursors. (A) FLK1+ cells were sorted from day 3.25 Sox7-Gfp embryoid bodies (EBs) and cultured in 2D culture. (B) Flow cytometry analysis of SOX7-GFP+ and SOX-GFP− fractions at day 2 of culture. Data are representative of 3 independent experiments. (C) QRT-PCR analysis for the expression of the indicated genes in sorted SOX7-GFP+ and SOX7-GFP− fractions at day 2 of the culture. Error bars indicate ± SEM (n = 3 independent experiments), Student’s paired two-tailed t-test. (D) E7.5 Sox7LacZ/WT embryos: (i) whole mount S-gal staining, (ii) hematoxylin and eosin (H&E) staining on section, (iii) S-gal and methyl green staining on section, (iv) close-up image of allantois from the S-gal staining, (v) close-up image of blood island from the S-gal staining. YS: yolk sac, EP: embryo proper, A: allantois, BL: blood island, PE: primitive endoderm. Scale bars: 500 μm. (E) E10.5 Sox7LacZ/WT embryos. Left panel: S-gal staining on a section from dorsal aorta. Right: Immunostaining on the following section. Red arrows indicate emerging hematopoietic clusters. Yellow asterisks indicate SOX7::S-gal− blood cells. Scale bars: 50 μm.
Here, we performed a detailed analysis of the vascular defects resulting from either a complete deficiency in Sox7 expression or from the conditional deletion of Sox7 in FLK1-expressing cells.

2. Materials and methods

2.1. ESC culture and differentiation

Embryonic stem cells (ESCs) were cultured and differentiated as previously described (Sroczynska et al., 2009). Embryoid bodies (EBs) were routinely maintained up to day 3, and FLK1+ cells were isolated and cultured as previously described (Fehling et al., 2003; Lancrin et al., 2009).

2.2. Generation of Sox7 knockout mouse lines

Targeted Sox7 ESC clone B9 (International Knockout Mouse Consortium) was injected into mouse blastocysts. Resultant chimaeras were crossed with C57BL/6 mice. Subsequent generations were crossed with PGK-Cre mice to excise the neomycin cassette and exon 2 of the Sox7 gene, resulting in the generation of a LacZ-flxed null allele (Sox7lacZ/WT). Alternatively to generate the Sox7-flxed allele, mice were crossed with an actin-FLP transgenic line resulting in the excision of both IRES-LacZ and neomycin cassettes that are flanked by FRT site (International Knockout Mouse Consortium). After eight backcrosses on C57BL/6, mice were either inter-crossed to generate Sox7fl/fl or crossed with Flk1-cre (Motoike et al., 2003) or Vav-Cre (de Boer et al., 2003) transgenic lines to excise in a tissue specific manner the exon 2 of Sox7 that is flanked by LoxP sites.

2.3. Timed matings

Timed matings were set up between: heterozygous male and female Sox7lacZ/WT mice, heterozygous Sox7fl/fl Flk1-cre male and Sox7fl/fl or Sox7fl/fl female mice. The morning of vaginal plug detection was embryonic day (E) 0.5. All animal work was performed under regulation governed by the Home Office Legislation under the Animal Scientific Procedures Act (ASPA) 1986.

2.4. QRT-PCR

Total RNA was isolated using Rneasy Mini/Micro plus Kit (Qiagen), and 2 μg of which was used to generate cDNA using the Omniscript reverse transcriptase kit (Qiagen), according to the manufacturer’s instructions. Real time PCR were performed on an ABI 7900 system (Applied Biosystems) using the Exiqon universal probe library (Roche). Gene expression was calculated relative to β-actin using the ΔΔCt method.

2.5. Whole mount and section staining

Embryos were stained using a rat anti-mouse CD31 antibody (1:500) (BD biosciences; 553,370) and a goat anti-rat AF555 secondary antibody (1:1000) (Invitrogen) as previously described (Yokomizo et al., 2012). Z-stack images were taken using a two-photon confocal microscope with a 5 × objective (Leica). E10.5 embryo sections were stained as previously described (Thambryrajah et al., 2016) using a goat anti-SOX7 antibody (1:200) (R&D systems; AF2766) and a donkey anti-goat AF647 antibody (1:2000) (Invitrogen). Subsequently, embryos were stained with a rat anti-cKit antibody (1:1000) (BD biosciences; 553,868), and a rabbit anti-pan-RUNX antibody (1:1000) (Abcam; ab92336) before staining with a goat anti-rat AF488 and a goat anti-rabbit antibody (both 1:2000) (both Invitrogen). Yolk sacs were isolated and flat mounted with DAPI as previously described (Frame et al., 2016) before imaging. Specific labelling of primary antibodies was determined by comparison with no primary antibody stained controls.

2.6. Flow cytometry

Cells were disaggregated by trypsinisation, and incubated with combinations of conjugated monoclonal antibodies on ice. Analyses were performed on a BD LSRII (BD Biosciences). Data were analysed with FlowJo (TreeStar), gating first on the forward scatter versus side scatter to exclude non-viable cells.

2.7. Statistical analyses

Sample sizes were chosen based on previous experimental experience. Student’s t-test was used to assess the differences between two populations in embryo experiments. * P-value < 0.05, **P-value < 0.01, *** P-value < 0.001.

3. Results and discussion

3.1. SOX7 is expressed in EPCs at the onset of endothelial specification from mesoderm

To define at the cellular level the expression of SOX7 during the earliest step of cardiovascular specification from mesoderm, we used...
an embryonic stem cell (ESC) line carrying a BAC transgene with the first exon of Sox7 replaced by a Gfp reporter cDNA (Gandillet et al., 2009). These ESCs were differentiated in vitro to mesoderm via embryoid body (EB) formation (Fehling et al., 2003). This differentiation process led to the generation of a FLK1+ mesoderm population that was isolated and further differentiated to a TIE2+ cKIT+ cell population containing both hemogenic endothelial and EPCs as previously described (Lancrin et al., 2009). FLK1+ cells sorted from Sox7-GFP EBs were cultured as a monolayer and analysed after 2 days of culture (Fig. 1A). The SOX7-GFP+ fraction was strongly enriched for the expression of the endothelial marker TIE2, VE-Cadherin and CD31, and to a lesser extent, for c-KIT when compared to the SOX7-GFP− fraction (Fig. 1B). Furthermore, the SOX7-GFP+ fraction had significantly higher transcript levels of Flk1, Gata2, and Scl genes, while there were also higher transcript levels of Fli1 and Cdh5 (Fig. 1C). Collectively, these data indicate that in vitro, SOX7 is expressed in a very large fraction EPCs at the onset of endothelial specification from mesodermal precursors.

To investigate the pattern of SOX7 expression during in vivo development, mouse embryos heterozygous for a Sox7-LacZ null allele were generated. Whole mount S-gal staining of E7.5 embryos revealed the widespread presence of SOX7:LACZ-expressing cells in the yolk sac region of the developing conceptus in agreement with previously published data (Gandillet et al., 2009) (Fig. 1D). Further S-gal staining of E7.5 embryo sections confirmed the expression of SOX7 in the blood islands, allantois and primitive endoderm (Fig. 1D). In E10.5 embryos, S-gal staining highlighted SOX7::LACZ-expressing cells in the endothelium lining of the dorsal aorta (Fig. 1E). Additionally, immunostaining

Fig. 3. SOX7 deficient embryos show defects in the dorsal aorta and vascular plexus of the yolk sac at E8.5. (A) Whole mount PECAM1 staining of heterozygous (Sox7+/−) and Sox7 knockout (Sox7−/−) embryos and yolk sacs at E8.5 (5–6 somite pairs), 3D projection of the embryo embedded within its yolk sac. White arrows indicate anterior (A), posterior (P), distal (D) and proximal (Pr) axes. (B) Anterior view of the dorsal aorta (white arrowheads). (C) Posterior tip of the dorsal aorta (yellow arrows). (D) Whole mount PECAM1 staining of Sox7+/− and Sox7−/− E8.5 embryos (3–5 somite pairs). The boxes denote area of magnification: (i) magnified view of dorsal aorta, red bar denotes cross section area, (ii) cross section of dorsal aorta lumen. (E) Details of the yolk sac vasculature. Scale bars: 100 μm. Data shown are representative of at least 3 embryos with 100% penetrance of the phenotype observed for knockout embryos.
revealed that CD31⁺ c-KIT⁺ hematopoietic clusters expressed SOX7 (Fig. 1E, red arrows) whereas few hematopoietic cells within the aortal lumen also expressed SOX7 (Fig. 1E, yellow asterisks). These data confirm that SOX7 is expressed in the blood islands during the emergence of the first EPCs, as well as at later stages in endothelial cells during vascular development. It is interesting to note that SOX7 also appears to be expressed in hemogenic endothelium of the dorsal aorta since emerging clusters of blood cells do express SOX7. Given the very early onset of Sox7 expression during the specification of the cardiovascular system, it is important to define how early during development this transcription factor is required for vasculogenesis and angiogenesis.

3.2. Sox7 complete knockout embryos have profound defects in vasculogenesis and angiogenesis

In order to elucidate the role of Sox7 during embryonic development, we first generated complete Sox7 knockout embryos (Sox7⁻/⁻) on homogenous genetic background by backcrossing Sox7lacZ/+ mice on C57Bl/6 then by inter-crossing these transgenic mice. The LacZ cassette was inserted at the beginning of exon 2 and therefore fully disrupts the expression of Sox7. Complete deficiency in Sox7 on this homogenous background led to a fully penetrant embryonic lethality phenotype by E10.5 characterised by severe growth retardation as well as an absence of large blood vessels in the yolk sac (Fig. 2A) as previously observed (Wat et al., 2012). To understand how these defects occurred, we investigated the formation of the vascular system prior to E10.5, a development time point at which Sox7 deficiency resulted in lethality in all embryos examined. Whole mount PECAM1 staining at E7.5 revealed that Sox7 deficiency did not affect the overall generation of PECAM1⁺ primordial EPCs (Fig. 2B). However, one day later by E8.5, Sox7⁻/⁻ embryos already displayed notable defects in the developing vascular networks which are formed by vasculogenesis (Fig. 3A). The development of the anterior region of the paired dorsal aorta was relatively unaffected (Fig. 3B, white arrowheads), but the posterior region displayed areas of highly unorganised endothelial cords rather than a distinct paired dorsal aorta (Fig. 3C, yellow arrows and Supplemental video 1). In addition, the posterior regions of the dorsal aorta were not lumenized in Sox7⁻/⁻ embryos (Fig. 3D). Finally, whilst a primitive vascular plexus formed within the yolk sac of Sox7⁻/⁻ embryos, the vascular network appeared disorganized compared to that of the control embryos (Fig. 3E).

By E10.5, whole mount PECAM1 staining revealed that Sox7 deficiency led to extremely severe vascular defects both in the embryo proper and in the yolk sac (Fig. 4A and B). In Sox7⁻/⁻ embryos there was an absence of a definitive dorsal aorta in the posterior region of the embryo (Fig. 4A, yellow arrows), indicating that the dorsal aorta did not recover from the initial vasculogenic defects observed at E8.5. Furthermore, the highly unorganised nature of the vascular networks indicates considerable angiogenic remodelling defects resulting from Sox7 deficiency. At E10.5, the yolk sac vasculature of Sox7⁻/⁻ embryos was arrested at the primitive vascular plexus stage, with a complete absence of vascular remodelling (Fig. 4B). Together these findings demonstrate that SOX7 is critically required for both vasculogenesis and vascular remodelling during angiogenesis. A detailed study of sprouting defect in the retina upon Cdh5-CreER induced deletion of Sox7 has recently been published by Kim et al. (Kim et al., 2016), suggesting that SOX7 is important for both remodelling and sprouting during angiogenesis.

Unlike other intra-embryonic vessels and arteries, the nascent dorsal aortae originate from paired lateral cords that are formed by the migration and aggregation of angioblasts (Drake and Fleming, 2000; Sato,
The fact that the posterior part of the dorsal aorta is affected in Sox7 deficient embryos strongly suggests an early defect in cord assembly at a vasculogenesis stage. It has been well characterised that there is redundancy and compensation between SOXF family members in controlling vascular development (Hosking et al., 2009; Matsui et al., 2006; Zhou et al., 2015). The conditional knockout of SOX7 in TIE2

**Fig. 6.** Flk1-Cre Sox7^{fl/fl} embryos have defects in the dorsal aorta at E8.5. Whole mount PECAM1 staining of control (Sox7^{fl/+}) and Flk1-Cre Sox7^{fl/fl} embryos and yolk sac at E8.5 (5–7 somite pairs). (A) 3D projection of embryos embedded within their yolk sacs, white arrows indicated anterior (A), posterior (P), distal (D) and proximal (Pr) axes. (B) Details of the yolk sac vasculature. (C) Paired dorsal aorta region. (D) Magnified view of a posterior region of a single dorsal aorta. Yellow arrows indicate malformation in the dorsal aorta. Scale bars 100 μm (A and C), 50 μm (B and D). Data are representative of at least three embryos with 100% penetrance of the phenotype observed for knockout embryos.
expressing endothelial cells using mice from a mixed genetic background, resulted in relatively minor vascular defects such as a decrease in the diameter of the dorsal aorta (Zhou et al., 2015). These relatively minor defects reported by Zhou and collaborators are most likely due to compensation by SOX17 and SOX18 rather than truly a result of \( \text{Sox7} \) deletion in TIE2-expressing cells. Indeed, it was shown that in \( \text{Sox18}^{-/-} \) mice of a mixed genetic background SOX7 and SOX17 were upregulated and substituted for SOX18 (Hosking et al., 2009). Given this known redundancy and compensation among the three SOXF genes, we examined transcript levels for \( \text{Sox17} \) and \( \text{Sox18} \) in \( \text{Sox7}^{-/-} \) embryos relative to \( \text{Sox7}^{+/+} \) embryos (Fig. 5). This analysis revealed an increase in the expression of both \( \text{Sox17} \) and \( \text{Sox18} \), suggesting possible compensation of SOX7 deficiency by SOX17 and SOX18. However, even with the compensatory activity by SOX17 and SOX18, Sox7 deficiency resulted in massive vasculogenic and angiogenic defects. Together these data support a critical and unique role for SOX7 in the development of the vascular system.

3.3. Sox7 conditional deletion in FLK1-expressing cells leads to severe defects in vasculogenesis and angiogenesis

In addition to its expression in endothelial progenitors, Sox7 has been previously detected in primitive endoderm (Futaki et al., 2004; Murakami et al., 2004), in the earliest specified hematopoietic progenitors (Gandillet et al., 2009) and in emerging hematopoietic clusters in the dorsal aorta (Lilly et al., 2016; Nobuhisa et al., 2014). To define a possible role and requirement for Sox7 in specific compartments, we generated a Sox7 conditional allele in which the exon 2 of Sox7 is flanked by LoxP sequences and can be excised upon CRE expression. First, we analysed the requirement for Sox7 expression in the vascular compartment using a \( \text{Flk1-cre} \) transgenic mouse line (Motoike et al., 2003). The conditional deletion of Sox7 in FLK1-expressing cells resulted in early embryonic lethality and a phenotype very similar to the complete Sox7 knockout embryos. At E8.5, whole mount PECAM1 staining revealed that \( \text{Flk1-Cre Sox7}^{-/-} \) embryos displayed already noticeable defects in the developing vascular networks.

---

**Fig. 7.** \( \text{Flk1-Cre Sox7}^{-/-} \) embryos have severe and widespread vascular defects at E10.5. Whole mount PECAM1 staining of Sox7 and \( \text{Flk1-Cre Sox7}^{-/-} \) embryos at E10.5. (A) 3D projection of embryo proper, white boxes indicate areas of magnification. Data shown are representative of 4 embryos. (B) Top panel: organization of capillaries in posterior region, bottom panel: vitelline artery (VA). (C) Sagittal slices through the embryo proper. DA: dorsal aorta, white arrow indicates length of functional dorsal aorta; yellow arrows indicate malformation of the DA. (D) Mean length of DA relative to embryo ± SEM, \( n = 6 \) control (Sox7 and \( \text{Sox7}^{-/-} \)) versus \( n = 4 \) \( \text{Flk1-Cre Sox7}^{-/-} \) embryos. (E) Expression of FLK1 and TIE2 in wild type gastrulating embryos at early streak (left panel) and late streak (right panel) stages measured by flow cytometry.
(Fig. 6A–D) including a disorganized yolk sac vascular plexus (Fig. 6B) and areas of highly unorganised endothelial cords in the posterior region of the dorsal aorta (Fig. 6C–D, yellow arrows). By E10.5, the highly unorganised nature of the vascular networks was indicative of considerable angiogenic defects resulting from the deletion of Sox7 in FLK1-expressing cells (Fig. 7A) in agreement with the recently published phenotype of the Tie2-specific Sox7 knockout embryos (Kim et al., 2016) that was performed on homogenous genetic background unlike the study by Zhou and collaborators (Zhou et al., 2015). In contrast to Tie2-specific Sox7 deletion, the endothelial Flk1-specific deletion of Sox7 revealed marked defects in the formation of the major blood vessels in the embryo indicative of vasculogenetic defects. In Flk1-Cre Sox7fl/fl embryos, there was a lack of an observable vitelline artery in the embryo proper (Fig. 7B, yellow arrows). Furthermore, the functional dorsal aorta in Flk1-Cre Sox7fl/fl embryos was extremely short, with the posterior region of the dorsal aorta resembling a cord of endothelial cells suggesting major defects in the vasculogenic events leading to the formation and organization of the angioblast cords giving rise to the posterior region of the dorsal aorta (Fig. 7C–D, white arrows). The lack of vitelline artery is most likely a direct consequence of the absence of the posterior dorsal aorta as the vitelline artery arises from the dorsal aorta (Drake and Fleming, 2000). It is likely that the differences between the Sox7 Tie2-deleted and Flk1-deleted embryonic phenotypes results from the earlier expression of FLK1 during development (Fig. 7E) and therefore the earlier deletion of Sox7 in Flk1-cre than in Tie2-cre embryos. Unlike Tie2, the expression of Flk1 is detected in mesoderm and mesenchyme (Ema et al., 2006), it is possible that Sox7 deletion in these tissues contributes to the stronger phenotype observed. These findings demonstrate that the expression of SOX7 is required earlier than previously described (Kim et al., 2016) and that SOX7 is an important transcriptional regulator of vasculogenesis.

To further analyse the vascular defects in Flk1-Cre Sox7fl/fl embryos, we performed PECAM1 staining on sections of E9.5 embryos (Fig. 8A and B). The complete disorganisation of the vascular network made comparisons of specific blood vessels with control embryos impossible. However, there was clear evidence of lumenization defects in a major blood vessel, as a mass of disorganized endothelial cells was observed (Fig. 8A, yellow arrow) that in the subsequent section formed a blood vessel with a lumen (Fig. 8B, red arrow). At E10.5 the yolk sac vasculature of Flk1-Cre Sox7fl/fl embryos was arrested at a primitive vascular plexus stage, with a complete absence of vascular remodelling (Fig. 9A). In control yolk sacs, venous and arterial areas were easily identified along with the vitelline vein (VV) and vitelline artery (VA); in contrast the yolk sac of Flk1-Cre Sox7fl/fl embryos only contained a homogenous plexus of vessels with relatively large diameters as shown by blood vessel diameter measurement (Fig. 9B). Furthermore, measurement of the space between capillaries identified that Flk1-Cre Sox7fl/fl plexuses have a decreased avascular space compared to capillaries of control yolk sacs (Fig. 9C). This is in contrast to embryos with hemodynamic flow deficiencies which show larger avascular space between non-remodelled yolk sac plexus blood vessels when compared to control embryos (Jones et al., 2008). Together, this suggests that the phenotype of the Flk1-Cre Sox7fl/fl embryos is largely endothelial based and not due to cardiac defects causing decreased blood flow. Taken together, these data demonstrate that SOX7 is critically required in FLK1-expressing cells for both vasculogenesis and angiogenesis. In particular, SOX7 seems to be critical for the formation of a fully lumenized dorsal aorta, suggestive
SOX17 compensates for SOX7 loss in primitive endoderm since SOX17 is expressed in the earliest blood progenitors, this transcription factor is not required for definitive hematopoiesis which encompasses all blood cells generated from E8.5 onward, including hematopoietic stem cells. However, it remains possible that the two other SOXF factors are providing enough compensation to allow for the emergence of blood cells in developing embryos deficient for Sox7 expression in Vav-expressing cells. The generation of triple Sox7 conditional embryos would be required to address this possibility.

3.4. Sox7 conditional deletion in VAV-expressing cells does not affect the hematopoietic system

Finally, we analysed the consequence of the specific deletion of Sox7 in the hematopoietic compartment given the observed expression of Sox7 at all sites of hematopoietic emergence during embryogenesis (Lilly et al., 2016). Indeed, this was further confirmed by the co-expression of SOX7 with RUNX and cKIT, both marking emerging blood cells in the ventral aspect of the dorsal aorta in wild type embryos at E10.5 (Fig. 10A). To determine a possible role of SOX7 in hematopoiesis, Sox7fl/fl mice were crossed with Vav-Cre transgenic mice (de Boer et al., 2003), which resulted in Sox7 deficiency in all definitive hematopoietic cells. Interestingly, Vav-Cre Sox7fl/fl pups were viable and lived to adulthood without any phenotypic abnormalities or observable defects in the hematopoietic system (Fig. 10B–E). These data demonstrate that while Sox7 is expressed in the earliest blood progenitors, this transcription factor is not required for definitive hematopoiesis which encompasses all blood cells generated from E8.5 onward, including hematopoietic stem cells. However, it remains possible that the two other SOXF factors are providing enough compensation to allow for the emergence of blood cells in developing embryos deficient for Sox7 expression in Vav-expressing cells. The generation of triple Sox7 conditional embryos would be required to address this possibility.

4. Concluding remark

The development of new vascular networks via vasculogenesis and angiogenesis is an important factor in the pathophysiology of all solid tumours. Neoplastic vascularisation facilitates the proliferation and subsequent metastasis of tumour cells, making angiogenic processes
attractive targets in combating cancer (Nishida et al., 2006). The role of SOX7 in promoting tumour progression and angiogenesis is poorly understood. Recent data suggest that SOX17 is an important regulator of tumour angiogenesis (Yang et al., 2013). Together, these findings warrant further investigation into whether SOXF factors act redundantly or compensate for each other to promote tumour angiogenesis, which may offer novel therapeutic targets for the treatment of cancer.

Conflict of interest

The authors declare no competing financial interests.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.mod.2017.05.004.

Acknowledgements

We thank the staff at the Flow Cytometry, Advanced Imaging and Molecular Biology Core facilities of CRUK Manchester Institute for technical support. Research in the authors’ laboratory is supported by the Medical Research Council (MR/P000673/1), the Biotechnology and Biological Sciences Research Council (BB/1001794/1) and Cancer Research UK (C5759/A20971).

References


