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The chromatin-remodeling enzyme BRG1 plays an essential role in primitive erythropoiesis and vascular development

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Abstract

ATP-dependent chromatin-remodeling complexes contribute to the proper temporal and spatial patterns of gene expression in mammalian embryos and therefore play important roles in a number of developmental processes. SWI/SNF-like chromatin-remodeling complexes use one of two different ATPases as their catalytic subunit: brahma (BRM, also known as SMARCA2) and brahma-related gene 1 (BRG1, also known as SMARCA4). We have conditionally deleted a floxed *Brg1* allele with a *Tie2-Cre* transgene, which is expressed in developing hematopoietic and endothelial cells. *Brg1*$^{fl/fl}$*:Tie2-Cre*$^{+}$ embryos die at midgestation from anemia, as mutant primitive erythrocytes fail to transcribe embryonic α-and β-globins, and subsequently undergo apoptosis. Additionally, vascular remodeling of the extraembryonic yolk sac is abnormal in *Brg1*$^{fl/fl}$*:Tie2-Cre*$^{+}$ embryos. Importantly, *Brm* deficiency does not exacerbate the erythropoietic or vascular abnormalities found in *Brg1*$^{fl/fl}$*:Tie2-Cre*$^{+}$ embryos, implying that *Brg1*-containing SWI/SNF-like complexes, rather than *Brm*-containing complexes, play a crucial role in primitive erythropoiesis and in early vascular development.

Keywords

SWI/SNF; Brg1; Tie2-Cre; Erythropoiesis; β-globin; Vascular remodeling; Angiogenesis

INTRODUCTION

Developmental processes require changes in gene expression to achieve cellular differentiation. Eukaryotes use chromatin-modifying factors to aid in the regulation of gene expression because large nuclear factors necessary for transcription cannot access DNA when it is tightly bound to histones in nucleosomes. Two main classes of chromatin-modifying factors achieve changes in chromatin structure and organization at individual genes. One class covalently modifies histone proteins to achieve a heritable epigenetic mark instructing further genetic regulation. The second class uses energy derived from ATP hydrolysis to alter the conformation or position of nucleosomes, thereby transiently making gene promoters accessible or inaccessible to large nuclear factors. Both classes of chromatin-modifying factors are necessary to achieve proper temporal and spatial patterns of gene expression in the embryo, and therefore play important roles in a number of developmental processes (de la Serna et al., 2006; Margueron et al., 2005).

The mammalian SWI/SNF-related chromatin-remodeling complexes comprise one major family of ATP-dependent chromatin-modifying factors. These large, multi-protein complexes use one of two different ATPases as their catalytic subunit: brahma (BRM, also known as

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SMARCA2) and brahma-related gene 1 (BRG1, also known as SMARCA4). The significance of SWI/SNF-related complexes in mammalian development is particularly well demonstrated by the phenotypes associated with mice carrying mutations in *Brg1*. *Brg1*$^{-/-}$ embryos die at the peri-implantation stage of development, and conditional alleles have been used to demonstrate the role of *Brg1* in T-cell development, limb morphogenesis, skin development, gliogenesis and zygotic genome activation (Bultman et al., 2000; Bultman et al., 2006; Gebuhr et al., 2003; Indra et al., 2005; Matsumoto et al., 2006). By contrast, *Brm*$^{-/-}$ mice develop normally, although adult mutants are 15% heavier than their control littermates, possibly because of increased cellular proliferation (Reyes et al., 1998). As BRG1 is significantly upregulated in *Brm*$^{-/-}$ mice, it has been hypothesized that BRG1 can functionally compensate for the loss of BRM during development (Reyes et al., 1998).

We previously isolated and characterized an *N*-ethyl-*N*-nitrosourea (ENU)-induced point mutation in *Brg1* (*Brg1*^{ENU1}) that changes a single amino-acid residue (E1083G) in a highly conserved region of the catalytic ATPase domain (Bultman et al., 2005). The mutant protein is stable, assembles into SWI/SNF-related complexes, and exhibits normal ATPase activity, but has diminished nucleosome-remodeling capability. *Brg1*^{null}/*Brg1*^{ENU1} embryos fail to transcribe adult β-globin genes, thereby indicating that BRG1 plays an important role in chromatin remodeling of the β-globin locus during definitive erythropoiesis. However, because hypomorphic *Brg1*^{null}/*Brg1*^{ENU1} embryos express embryonic β-globin genes and undergo normal primitive erythropoiesis, it has been unclear whether BRG1 and SWI/SNF-related complexes are involved in this earlier hematopoietic process. Using a conditional null allele, we now report that BRG1, but not BRM, is indeed recruited to the β-globin locus control region in primitive erythrocytes, and is required for the transcription of embryonic β-globin genes and for erythroblast survival. We also demonstrate for the first time that BRG1 is required for embryonic α-globin expression, although expression of adult α-globins does not depend upon BRG1-induced remodeling in primitive erythrocytes. Finally, BRG1 appears to play an important role in vascular development that is separable from its role in primitive erythropoiesis.

MATERIALS AND METHODS

Mice

The *Brg1*-floxed mice (Gebuhr et al., 2003), the *Brm*$^{-/-}$ mice (Reyes et al., 1998), the *Tie2-Cre* transgenic mice (Koni et al., 2001) and the *ROSA26R* mice (Soriano, 1999) have been described. All mice were maintained on a mixed genetic background at the University of North Carolina, Chapel Hill Animal Facility. *Brg1*-floxed mice were genotyped by Southern blot analysis using a 449 bp probe against the 3′ portion of intron 14 and genomic DNA digested with *Bam*HI. The PCR primers used to generate the Southern probe were as follows: forward, 5′-TGGCATCTCATTTGTGTGGT-3′; and reverse, 5′-ACAGCCACTGGTTAGGGATG-3′. The Southern blot yields a 7.7 kb floxed allele, a 6.0 kb wild-type allele and a 5.5 kb excised allele. *Brg1*-floxed embryos were genotyped by PCR using the following primers: forward, 5′-GTCATACTTATGTCATAGCC-3′; and reverse, 5′-GCCTTGTCTCAAACTGATAAG-3′. These primers flank the 3′ LoxP site, and yield a 387 bp floxed allele and a 230 bp wild-type allele. The PCR was performed at an annealing temperature of 51°C. *Brm*$^{-/-}$ mice and embryos were genotyped by PCR using the following primers for the 197 bp wild-type allele: forward, 5′-ATATCTGGAGGAGGCCCAAC-3′; and reverse, 5′-TGCAGAGTTTCAGGGAGAGG-3′. The 600 bp targeted allele was amplified using the same forward primer and the following reverse primer: 5′-CATCGCCTTCTATCGCCTTC-3′. The PCR was performed at an annealing temperature of 55°C. *Tie2-Cre* transgenic mice and embryos were genotyped using a gene-specific forward primer (5′-GGGAAGTCGCAAAGTTGTGAGTT-3′) and a Cre-specific reverse primer (5′

GTGAAACAGCATTGCTGTCACTT-3′) that amplifies a 400 bp product. Control primers amplifying a 324 bp product from the *IL2* gene were used as a template control: forward, 5′-CTAGGCCACAGAATTGAAAGATCT-3′; and reverse, 5′-GTAGGTGGAAATTCTAGCATCATCC-3′. The PCR was performed at an annealing temperature of 51°C. ROSA26R mice were genotyped as described (Soriano, 1999). All histological sections were scraped from paraffin or OCT (Tissue-Tek) into DEXPAT Reagent (TaKaRa) before genotyping.

Histology

Embryos and yolk sacs were dissected from maternal tissue, immersion-fixed in 4% paraformaldehyde overnight, dehydrated, embedded in paraffin, sectioned (10 μm), and stained with hematoxylin and eosin. For cryosections, embryos were dissected, fixed, and passed through 10% sucrose (10 minutes), 15% sucrose (10 minutes), 20% sucrose (1 hour) and 20% sucrose/OCT (overnight), and then embedded in OCT on dry ice before sectioning (10 μm) and mounting on Superfrost/Plus microscope slides (Fisher). Electron microscopy was performed as described (Schwarz et al., 2002) on embryonic day 10.5 (E10.5) yolk sacs from two mutant and two control littermate embryos with visible heart beats.

Staining

Whole-mount immunostaining for platelet/endothelial cell adhesion molecule 1 (PECAM1) was performed as described using a rat anti-mouse PECAM1 monoclonal antibody (BD PharMingen) (Schlaeger et al., 1995). Whole-mount β-galactosidase staining was performed as described (Schlaeger et al., 1995), and stained embryos were subsequently cryosectioned and counterstained with Nuclear Fast Red. Transferase-mediated deoxyuridine triphosphate (dUTP) nick-end labeling (TUNEL) staining was performed on paraffin-embedded tissues using the In Situ Cell Death Detection Kit, Fluorescein (Roche), according to the manufacturer's instructions. Benzidine staining was performed on cryosectioned embryos and yolk sacs. After a brief submersion in PBS, slides were incubated in methanol (15 seconds), 1% benzidine (Sigma-Aldrich) in methanol (5 minutes), 2.5% hydrogen peroxide in 70% ethanol (2.5 minutes), and washed in water (2.5 minutes).

In situ hybridization

Gene-specific antisense probes to the murine *εy*, *βH1*, *ζ* and *α1/2* globins have been described (Kingsley et al., 2006). A 382 bp antisense probe for murine *Band3* was amplified from E8.5 yolk sac cDNA using the following primers: forward, 5′-AGAGACCTAACCATCCCTGTGA-3′; and reverse, 5′-TCTGATCCTCGTAGATGAAGCA-3′. A 425 bp antisense probe for murine *Alas2* was amplified from E8.5 yolk sac cDNA using the following primers: forward, 5′-CCATGCTGTAGGACTGTATGGA-3′; and reverse, 5′-CATAGATGCTGTGCTTGGAGAG-3′. In vitro transcription was performed to generate digoxigenin-labeled RNA probes. Cryosectioned embryos were subjected to a 10-minute proteinase K (2.5 μg/ml) pre-treatment before in situ hybridization. Prehybridization and hybridization incubations were carried out at 60°C in a mixture of 50% formamide, 5×SSC, 2% blocking reagent (Roche), 0.1% Triton-X100, 0.5% CHAPS, 50 μg/ml yeast tRNA, 5 mM EDTA and 100 μg/ml heparin. After overnight hybridization, slides were washed and incubated with anti-digoxigenin-AP Fab fragments (Roche) overnight at 4°C. After further washing, slides were incubated with NBT/BCIP at room temperature for several hours, or at 4°C for up to three days.

Chromatin immunoprecipitation (ChIP)

To obtain primitive erythrocytes, approximately 40 wild-type E9.5-E10.5 embryos were separated from their placentae by severing the umbilical vessels. The embryos with their attached yolk sacs were immediately placed in minimal essential media containing 2% fetal bovine serum (JRH Biosciences) and were rocked on a Nutator mixer for approximately 30 minutes at room temperature while allowing the hearts to pump the majority of circulating blood out of the embryos and yolk sacs. Embryos and yolk sacs were removed from the media, and the remaining blood cells were counted on a hemocytometer. Typically 20×10^6 to 50×10^6 blood cells were collected. The fresh cells were used in ChIP assays as previously described (Bultman et al., 2005), with some modifications: 7×10^6 cells were used for each ChIP or mock reaction; 0.6% formaldehyde was used to cross-link the protein-protein and protein-DNA interactions; and chromatin was sonicated with eleven 10-second pulses at 10% maximum power on a Branson Digital Sonifier. The anti-BRG1 antibody J1 (a gift from G. Crabtree and W. Wang, Stanford University) and the anti-acetyl-histone 3 antibody (Santa Cruz, 06-599) were used for immunoprecipitations. The β-globin locus control region DNAseI hypersensitive site 3 (HS3) primers that were used for amplification of the ChIP products and controls were as follows: forward, 5′-AGGCCTCCTAGGGACTGAGA-3′; and reverse, 5′-AGACTCCACCCTGAGCTGAA-3′. The 158 bp product was amplified at an annealing temperature of 55°C for 35 cycles. The ζ amplicon spans 271bp of the promoter starting 535 bp upstream of the start site, and the PCR primers were as follows: forward, 5′-TATGGAGGGCTAGCTGGAGA-3′; and reverse, 5′-GGCCTTAGTCCCACACAGAA-3′. The product was amplified at an annealing temperature of 55°C for 34 cycles. The *α1/2* primers used for ChIP amplification were as follows: forward, 5′-GGGAGGAGACAGTGGACAAA-3′; and reverse, 5′-AGTGATGGCAGTTTGGGAAG-3′. The amplicon spans 257 bp of the promoter starting 515 bp upstream of the *α1* start site, and was amplified at an annealing temperature of 55°C for 30 cycles. Cycle numbers were determined for each amplicon based on the maximum amount of amplification that could be achieved before background bands appeared in the mock (no antibody) control lane.

Image acquisition

Brightfield histological images were obtained with a Leica DM IRB microscope (Leica Microsystems) using 10× (NA 0.25) and 20× (NA 0.4) objectives with a 1.5× magnification changer and a SPOT RT-Slider digital camera (Diagnostic Instruments). Images were acquired with SPOT RT Software version 4.5 (Diagnostic Instruments) and were globally optimized for brightness/contrast, if necessary, using Photoshop software (Adobe). Whole yolk sacs were imaged on a Leica MZ FLIII microscope (Leica Microsystems) using the camera and acquisition software described above. Fluorescent TUNEL images were obtained with a Leica DM LB Microscope (Leica Microsystems) using a 40× (NA 0.7) objective and a Retiga-2000R digital camera (QImaging). Black and white images were acquired with QCapture Software version 3.0 (QImaging), and were pseudocolored and merged with SPOT RT Software.

RESULTS

Brg1$^{fl/fl}$:Tie2-Cre$^{+/0}$ embryos die during development

We initially analyzed mice carrying a conditionally floxed allele of *Brg1* in combination with a *Tie2-Cre* recombinase transgene, which is expressed in developing hematopoietic and endothelial cells (Koni et al., 2001). We recovered no live *Brg1$^{fl/fl}$:Tie2-Cre$^{+/0}$* offspring from matings between *Brg1$^{fl/fl}$* and *Brg1$^{fl/+}$:Tie2-Cre$^{+/0}$* mice (Table 1), indicating that expression of *Brg1* on *Tie2+* cells is critical for embryonic development. These data also indicate that BRM does not compensate for loss of BRG1 on *Tie2+* cells, despite the observation that BRG1 and BRM can play partially redundant roles in vitro (Chiba et al., 1994; Phelan et al., 1999; Strobeck et al., 2002).

Further evidence that BRM is not functionally redundant with BRG1 in developing endothelium and hematopoietic cells comes from our observation that ablation of *Brm* does not result in any detectable phenotype on a $Brg1^{fl/+}$:*Tie2-Cre*$^{+/0}$ background (Table 2). Lethality of $Brm^{-/-}$;$Brg1^{fl/+}$:*Tie2-Cre*$^{+/0}$ embryos might have been predicted if a threshold level of *Brg1* and *Brm* were required in *Tie2*$^{+}$ cells during development. However $Brm^{-/-}$;$Brg1^{fl/+}$:*Tie2-Cre*$^{+/0}$ progeny were recovered from crosses between $Brm^{-/-}$;$Brg1^{fl/fl}$ and $Brm^{-/-}$;$Brg1^{fl/+}$:*Tie2-Cre*$^{+/0}$ mice in expected numbers. Together, our genetic data clearly illustrate the importance of *Brg1* over *Brm* in hematopoietic and/or vascular development.

$Brg1^{fl/fl}$:*Tie2-Cre*$^{+/0}$ blood cells undergo apoptosis

In order to determine the time of death of $Brg1^{fl/fl}$:*Tie2-Cre*$^{+/0}$ embryos, we performed dissections on E8.5-E12.5 embryos. We chose midgestation for our initial analysis as many mutants with hematopoietic or vascular defects die at this developmental stage (Coultas et al., 2005; Orkin and Zon, 1997). We determined that $Brg1^{fl/fl}$:*Tie2-Cre*$^{+/0}$ embryos die at E10.5-E11.0, based on the absence of a visible heartbeat and the onset of necrosis. By E9.5, mutant embryos are visibly paler than their control littermates, and mutant yolk sacs lack the large, blood-filled vitelline vessels that are readily visible in control yolk sacs. Importantly, no exacerbation of the timing or severity of this gross mutant phenotype is detected on a $Brm^{-/-}$ background (11 $Brm^{-/-}$;$Brg1^{fl/fl}$:*Tie2-Cre*$^{+/0}$ embryos were assessed at E9.5-E10.5), further demonstrating the importance of *Brg1* over *Brm* in hematopoietic and/or vascular development.

The extreme pallor of mutant embryos at E9.5 cannot be explained by hemorrhage. Upon gross dissection, no embryonic blood is visibly pooled in the extravascular exocoelomic, amniotic or pericardial cavities of mutant embryos. Likewise, histological analysis of control and mutant embryos from 19 litters (E8.5-E10.5), embedded intact within the maternal uterus so as not to disrupt any sites of pooled embryonic blood, failed to reveal any sign of hemorrhage.

The extraembryonic yolk sac is the initial site of hematopoiesis and produces large, nucleated blood cells that begin to circulate between the yolk sac and embryo at E8.25 (McGrath et al., 2003). Such blood cells are abundant in histological sections of E8.5 $Brg1^{fl/fl}$:*Tie2-Cre*$^{+/0}$ yolk sacs, demonstrating that hematopoiesis is unimpaired (Fig. 1B). However, by light and electron microscopy, a dramatic scarcity of blood cells is found in mutant embryos and yolk sacs at E9.5 and E10.5 (Fig. 1D). Furthermore, many of those blood cells that remain are fragmented and show abnormal membrane or nuclear morphology (Fig. 1F). In order to determine whether embryonic blood cells undergo apoptosis between E8.5 and E9.5, we performed TUNEL staining on sections of E9.5 mutant and control littermate embryos and yolk sacs. Blood cells from mutant embryos show more evidence of TUNEL staining than do those from control embryos (13.6±0.75% versus 0.4±0.2%, mean±s.e.m.), indicating that blood from $Brg1^{fl/fl}$:*Tie2-Cre*$^{+/0}$ embryos is subject to aberrant apoptosis (Fig. 2).

To determine whether the blood cell death detected in mutant embryos is cell autonomous, we crossed $Brg1^{fl/fl}$:*Tie2-Cre*$^{+/0}$ embryos onto the ROSA26R mouse line (Soriano, 1999). ROSA26R mice express β-galactosidase upon Cre-mediated deletion of a 'STOP' signal; therefore, cells that express *Tie2-Cre* turn blue upon X-gal staining in the presence of this reporter line. At E8.5, both control and mutant embryos display blue endothelium and comparable amounts of blue blood cells in their yolk sac vasculature (Fig. 3A,B,E). These data indicate that the majority of embryonic blood cells express *Tie2-Cre* at E8.5 and that *Brg1* is not required for production of these cells. At E9.5, both control and mutant embryos continue to display blue endothelium, but whereas control embryos reveal a preponderance of circulating blue blood cells (86±3.9%), mutants (which contain fewer blood cells overall) display a minority of blue blood cells (33.5±11.5%; Fig. 3C-E). Additionally, many of the blue blood cells in mutants are fragmented or display abnormal morphology (Fig. 3D, inset), whereas the non-blue blood cells retain normal morphology. This indicates that blood cells undergoing

Brg1 excision are selectively destroyed in a cell-autonomous fashion while wild-type blood cells are spared from destruction. Therefore, we conclude that *Brg1*$^{fl/fl}$*:Tie2-Cre*$^{+/0}$ blood cells are initially formed but subsequently undergo apoptosis between E8.5 and E10.5.

Brg1$^{fl/fl}$*:Tie2-Cre*$^{+/0}$ blood cells have defective embryonic globin transcription

The majority of circulating blood cells at E9.5 are large, nucleated erythroblasts, which are generated in yolk sac blood islands between E7.25-E9.0 (Palis et al., 1999; Wong et al., 1986). Once formed, these 'primitive' erythroblasts continue to divide for several days before entering the blood stream, undergoing maturation, and eventually enucleating (Kingsley et al., 2004). 'Definitive' erythroblasts, which originate in the yolk sac, placenta and aorta/gonad/mesonephros region, colonize the fetal liver where they expand and differentiate before entering the blood stream as mature, enucleated erythrocytes at E11.5-E12.5 (reviewed by McGrath and Palis, 2005; Mikkola et al., 2005). Definitive erythrocytes are the predominant cell type in the embryonic circulation after E12.5.

As they mature, both primitive and definitive erythroblasts accumulate hemoglobin, the oxygen-carrying component of red blood cells. In order to assess the production of hemoglobin in *Brg1*$^{fl/fl}$*:Tie2-Cre*$^{+/0}$ embryonic blood cells, we stained histological sections of control and mutant E9.5 embryos with benzidine (see Fig. S1 in the supplementary material). Although blood cells in control embryos exhibit strong staining, many blood cells in mutant embryos show little or no benzidine staining. These data indicate that hemoglobin production and/or accumulation is defective in *Brg1*$^{fl/fl}$*:Tie2-Cre*$^{+/0}$ primitive erythrocytes.

Globin tetramers encoded by the α-and β-globin gene loci are critical components of hemoglobin. The globin loci are multi-gene clusters: in mice there are three functional α-globins (*ζ*, *α1* and *α2*) and four functional β-globins (*εy*, *βH1*, *β*^{maj} and *β*^{min}). Whereas primitive erythroblasts express all of the globin genes, definitive erythrocytes express only the adult globins (*α1*, *α2*, *β*^{maj} and *β*^{min}) (Trimborn et al., 1999). Therefore a transition occurs during development (E10.5-E13.5) in which embryonic globin expression diminishes and adult globin expression escalates.

Brg1 is recruited to the β-globin locus control region (LCR), a long-distance upstream regulatory element, by erythroid-specific transcription factors, where it mediates chromatin remodeling to allow transcription of β-globin genes in vitro (Kadam et al., 2000). We hypothesized that the hemoglobin deficit observed in *Brg1*$^{fl/fl}$*:Tie2-Cre*$^{+/0}$ embryos could result from inadequate globin transcription. Because of the mixed population of wild-type and mutant blood cells in *Brg1*$^{fl/fl}$*:Tie2-Cre*$^{+/0}$ embryos at E9.5 (see Fig. 3D), we could not analyze the expression of α-globin and β-globin genes by RT-PCR. Instead, we performed in situ hybridization on sectioned embryos to visualize globin expression on a cell-by-cell basis. Control embryos express all of the predominant globins expected to be detected at E9.5 in the vast majority of their blood cells (Fig. 4A,C,E,G,I). Likewise, the adult α-globins (*α1* and *α2*) are expressed in almost every blood cell in mutant embryos at E9.5 (91.5±2.5%; Fig. 4D,I). However, the embryonic globins *ζ*, *εy* and *βH1* are only expressed in a subset of mutant blood cells at E9.5 (63.75±8.6%, 57.25±7.8% and 42.9±6%, respectively; Fig. 4B,F,H,I).

Our laboratory previously showed that BRG1 is recruited to the β-globin LCR in definitive erythrocytes in vivo, where it plays a crucial role in mediating adult β-globin transcription (Bultman et al., 2005). To demonstrate that BRG1 is also recruited to the β-globin LCR in primitive erythrocytes, we performed chromatin immunoprecipitation (ChIP) assays on circulating blood cells collected from E9.5-E10.5 embryos. These cells are predominantly primitive erythrocytes, as definitive erythrocytes first enter the bloodstream between E11.5-E12.5 (Brotherton et al., 1979; Kingsley et al., 2004). Using an anti-BRG1 antibody, we were able to demonstrate BRG1 binding to the DNAse I hypersensitive site 3 (HS3) of the β-globin

LCR, where it is recruited in definitive erythrocytes as well (Fig. 4J). These ChIP data indicate that the defects we detect in embryonic β-globin expression by in situ hybridization directly result from loss of BRG1-induced chromatin remodeling at the β-globin LCR in mutant primitive erythrocytes. Furthermore, we were able to detect BRG1 binding at the promoter of the embryonic α-globin ζ, whereas BRG1 does not bind the promoter of the adult α-globins α*1/2* (Fig. 4J). These ChIP results for the α-globin genes are consistent with our in situ hybridization results, which indicate BRG1 involvement in ζ but not α*1/2* expression at E9.5. Therefore, we provide the first evidence that BRG1 is important for embryonic α-globin transcription in primitive erythrocytes. Overall, the reduction in blood cells expressing embryonic globins, and the reduction in blood cells expressing *Tie2-Cre* by reporter analysis in E9.5 mutant embryos (see Fig. 3D,E), lead us to hypothesize that primitive erythrocytes having undergone excision of *Brg1* are unable to support normal levels of embryonic globin transcription, resulting in their destruction through apoptosis.

Vascular remodeling is abnormal in *Brg1$^{fl/fl}$:Tie2-Cre$^{+/0}$* yolk sacs

Because *Tie2-Cre* is expressed in developing endothelium as well as in hematopoietic cells, we analyzed the vasculature of *Brg1$^{fl/fl}$:Tie2-Cre$^{+/0}$* embryos by immunostaining embryos and yolk sacs with an antibody against the endothelial cell marker PECAM1. Yolk sac vascular development consists of two major steps: vasculogenesis, the process by which new blood vessels arise and form a primitive vascular plexus; and angiogenesis, the process by which the homogenous plexus undergoes vascular remodeling resulting in a hierarchical vascular tree comprising large and small vessels (Sato and Loughna, 2002). By E8.5, the time at which the primitive vascular plexus is formed, *Tie2-Cre*-mediated excision occurs efficiently in yolk sac endothelium (Fig. 3A,B). Yet *Brg1$^{fl/fl}$:Tie2-Cre$^{+/0}$* yolk sac vasculature is indistinguishable from that seen in control yolk sacs at this time (Fig. 5A,B), demonstrating that the vascular plexus formation/maintenance stages of vasculogenesis proceed normally in mutant yolk sacs. By E9.5, however, *Brg1$^{fl/fl}$:Tie2-Cre$^{+/0}$* yolk sacs demonstrate an aborted vascular remodeling process (Fig. 5D,E). Whereas their littermate controls successfully undergo angiogenesis, resulting in yolk sacs with interconnected large and small vessels, mutant yolk sacs progress beyond the plexus stage of vascular development but fail to establish a mature vascular tree. Instead, the mutant yolk sac vasculature consists of small and medium-sized vessels that occasionally fail to interconnect. Many dead-end or tapering vascular termini are visible in the mutant yolk sacs, indicating that vascular sprouting or pruning fails to progress normally (Fig. 5E). Dysregulated cardiac function and blood flow are unlikely causes of these vascular abnormalities because heart beat rates are normal and pericardial edema is rarely detected in *Brg1$^{fl/fl}$:Tie2-Cre$^{+/0}$* embryos at E9.5 when the yolk sac vascular abnormalities are readily apparent. Interestingly, PECAM1 immunostaining of the vasculature within the mutant embryo proper appears normal and comparable to that seen in control littermates or stage-matched embryos at E9.5 (data not shown). Therefore, the vascular abnormalities we observe on the *Brg1$^{fl/fl}$:Tie2-Cre$^{+/0}$* background are specific to the yolk sac. Finally, although BRM expression in the yolk sac is restricted to vascular endothelium (Dauvillier et al., 2001), we did not see any exacerbation of the *Brg1$^{fl/fl}$:Tie2-Cre$^{+/0}$* vascular phenotype on a *Brm$^{-/-}$* background. Therefore BRM does not appear to play a functionally redundant role with BRG1 during early vascular development.

DISCUSSION

BRG1 is one of two alternative catalytic subunits found in mammalian SWI/SNF-like chromatin-remodeling complexes, which are important transcriptional regulators during embryonic development. We provide the first evidence of a role for *Brg1* in embryonic β-globin expression and in primitive erythropoiesis in vivo. The phenotype of our conditional null mutants is more severe than that of hypomorphic *Brg1$^{null/ENU1}$* embryos (Bultman et al.,

2005). Erythroblasts in *Brg1*$^{null/ENU1}$ embryos contain a stable BRG1 protein that assembles into SWI/SNF-like complexes and retains normal ATPase activity. However, although the mutant BRG1^{ENU1} protein is effectively recruited to the β-globin LCR, as demonstrated by ChIP assays, the LCR does not establish DNAse I hypersensitive sites characteristic of open chromatin. Because of this closed chromatin configuration at the LCR, adult β-globin transcription is reduced in *Brg1*$^{null/ENU1}$ embryos, and definitive erythroblasts undergo developmental arrest, leading to embryonic lethality by E14.5. By contrast, *Brg1*$^{fl/fl}$*:Tie2-Cre*$^{+/0}$ erythroblasts contain little or no BRG1 protein, and fail to transcribe embryonic β-globins during primitive erythropoiesis, resulting in embryonic lethality by E11.0. Together, these hypomorphic and conditional null mutations demonstrate the requirement for *Brg1*-mediated chromatin remodeling during both embryonic and adult β-globin transcription.

Like *Brg1*$^{fl/fl}$*:Tie2-Cre*$^{+/0}$ mutants, mice targeted for deletion of the Krüppel-like factor 2 (*Klf2*) have a significant reduction of embryonic β-globin gene expression and an upregulation of apoptosis in primitive erythrocytes (Basu et al., 2005). However, unlike *Brg1*$^{fl/fl}$*:Tie2-Cre*$^{+/0}$ mutants, which die at E10.5-E11.0, *Klf2*$^{-/-}$ embryos persist until E12.5-E14.0, when they succumb to heart failure (Lee et al., 2006). Notably, *Klf2* is dispensable for adult β-globin gene expression in definitive erythrocytes (Basu et al., 2005). Perhaps *Brg1*$^{fl/fl}$*:Tie2-Cre*$^{+/0}$ mutants die at E10.5 from anemia whereas *Klf2*$^{-/-}$ embryos do not become anemic, because *Brg1* is necessary for transcription of both embryonic and adult β-globins whereas *Klf2* is only critical for embryonic β-globin transcription. We hypothesize that embryonic β-globins are required for the survival of primitive erythrocytes, but that definitive erythrocytes, which express adult β-globins, can compensate for loss of primitive erythrocytes at the primitive/definitive transition. This hypothesis is substantiated by the observation that mice depleted of both of the embryonic β-globins survive development whereas embryos depleted of all of the embryonic and adult β-globins, as a result of targeted deletion of the β-globin locus control region, die early in embryogenesis (Bender et al., 2000; Hu et al., 2007).

In addition to deficits in embryonic β-globin transcription, we also observe a reduction in embryonic α-globin (ζ) transcription in *Brg1*$^{fl/fl}$*:Tie2-Cre*$^{+/0}$ erythroblasts, although adult α-globin transcription occurs normally in these cells (Fig. 4). Furthermore, we demonstrate through ChIP assays that BRG1 binds the promoter of ζ but not the promoter of the adult α-globins. These data indicate that BRG1 facilitates α-globin expression at the individual gene promoters rather than at the major regulatory element (MRE), which is a long-distance upstream regulatory element comparable to the β-globin LCR. In this regard, the regulation of α-globin and β-globin expression by SWI/SNF-mediated chromatin remodeling appear to be distinct in primitive erythrocytes. β-Thalassemia occurs when a reduced synthesis of β-globin chains leads to an excessive accumulation of insoluble α-globin chains in red blood cells. The resulting blood cells have morphological abnormalities (reminiscent of those seen in Fig. 1F and Fig. 3D), and are vulnerable to mechanical injury and death. Although embryonic α-globin expression is compromised in *Brg1*$^{fl/fl}$*:Tie2-Cre*$^{+/0}$ erythroblasts, the adult α-globins are expressed normally and should be able to serve the survival needs of an embryo when β-globin chains are available (Leder et al., 1997). However, because of defective β-globin transcription in our mutant blood cells, the adult α-globins have no binding partners and presumably accumulate detrimentally. Therefore, we provide the first demonstration that loss of *Brg1* in primitive erythroblasts leads to severe β-thalassemia and lethality.

Because SWI/SNF might be expected to mediate transcription of multiple genes during erythropoiesis, we assessed expression of two other markers of erythroid development: *Alas2*, an important enzyme in heme biosynthesis; and *Band3* (*Slc4a1*), an integral membrane protein on the surface of erythrocytes that provides cellular mechanical stability. By in situ hybridization, we found normal expression of *Alas2* but deficient expression of *Band3* in *Brg1*$^{fl/fl}$*:Tie2-Cre*$^{+/0}$ erythroblasts (see Fig. S2 in the supplementary material), indicating that

BRG1-mediated chromatin remodeling may be important for *Band3* transcription. Because *Band3*$^{-/-}$ mice survive development (Peters et al., 1996;Southgate et al., 1996), we do not believe that aberrant *Band3* expression contributes to the lethality we observe in *Brg1*$^{fl/fl}$*:Tie2-Cre*$^{+/0}$ embryos. Nevertheless, in combination with the globin expression data presented here, these expression analyses provide interesting evidence for the specificity of SWI/SNF activity. Altogether, we demonstrate changes in the expression of embryonic globins and *Band3*, but no changes in the expression of adult α-globins or *Alas2* in our mutant erythroblasts. These data support a growing body of evidence that SWI/SNF complexes control a limited number of targets and are not simply indiscriminate transcriptional regulators (reviewed by Kwon and Wagner, 2007). As such, these and other chromatin remodeling complexes have the capacity to regulate a wide variety of nuanced developmental processes.

Finally, we believe *Brg1*$^{fl/fl}$*:Tie2-Cre*$^{+/0}$ embryonic lethality is due to anemia resulting from failure of hemoglobin synthesis and subsequent apoptosis of red blood cells, because other mutants with defective primitive erythropoiesis, such as *Gata1*, *Gata2* and *Rbtn2* mutants, all die from anemia at the same time in development as *Brg1*$^{fl/fl}$*:Tie2-Cre*$^{+/0}$ embryos (E10-E11.5) (Fujiwara et al., 1996; Tsai et al., 1994; Warren et al., 1994). Importantly, these mutants with defects in primitive erythropoiesis do not harbor secondary vascular defects. Therefore, we suspect that the vascular phenotypes we observe in *Brg1*$^{fl/fl}$*:Tie2-Cre*$^{+/0}$ mutant yolk sacs are separable from the mutant blood phenotype. We plan to verify this hypothesis in the future by rescuing expression of *Brg1* in developing erythrocytes and determining whether the vascular abnormalities in *Brg1*$^{fl/fl}$*:Tie2-Cre*$^{+/0}$ yolk sacs are still apparent at E9.5.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

Basu P, Morris PE, Haar JL, Wani MA, Lingrel JB, Gaensler KM, Lloyd JA. KLF2 is essential for primitive erythropoiesis and regulates the human and murine embryonic beta-like globin genes in vivo. Blood 2005;106:2566–2571. [PubMed: 15947087]

Bender MA, Bulger M, Close J, Groudine M. Beta-globin gene switching and DNase I sensitivity of the endogenous beta-globin locus in mice do not require the locus control region. Mol. Cell 2000;5:387–393. [PubMed: 10882079]

Brotherton TW, Chui DH, Gauldie J, Patterson M. Hemoglobin ontogeny during normal mouse fetal development. Proc. Natl. Acad. Sci. USA 1979;76:2853–2857. [PubMed: 111244]

Bultman S, Gebuhr T, Yee D, La Mantia C, Nicholson J, Gilliam A, Randazzo F, Metzger D, Chambon P, Crabtree G, et al. A Brg1 null mutation in the mouse reveals functional differences among mammalian SWI/SNF complexes. Mol. Cell 2000;6:1287–1295. [PubMed: 11163203]

Bultman SJ, Gebuhr TC, Magnuson T. A Brg1 mutation that uncouples ATPase activity from chromatin remodeling reveals an essential role for SWI/SNF-related complexes in beta-globin expression and erythroid development. Genes Dev 2005;19:2849–2861. [PubMed: 16287714]

Bultman SJ, Gebuhr TC, Pan H, Svoboda P, Schultz RM, Magnuson T. Maternal BRG1 regulates zygotic genome activation in the mouse. Genes Dev 2006;20:1744–1754. [PubMed: 16818606]

Chiba H, Muramatsu M, Nomoto A, Kato H. Two human homologues of Saccharomyces cerevisiae SWI2/SNF2 and Drosophila brahma are transcriptional coactivators cooperating with the estrogen receptor and the retinoic acid receptor. Nucleic Acids Res 1994;22:1815–1820. [PubMed: 8208605]

Coultas L, Chawengsaksophak K, Rossant J. Endothelial cells and VEGF in vascular development. Nature 2005;438:937–945. [PubMed: 16355211]

Dauvillier S, Ott MO, Renard JP, Legouy E. BRM (SNF2alpha) expression is concomitant to the onset of vasculogenesis in early mouse postimplantation development. Mech. Dev 2001;101:221–225. [PubMed: 11231080]

de la Serna IL, Ohkawa Y, Imbalzano AN. Chromatin remodelling in mammalian differentiation: lessons from ATP-dependent remodellers. Nat. Rev. Genet 2006;7:461–473. [PubMed: 16708073]

Fujiwara Y, Browne CP, Cunniff K, Goff SC, Orkin SH. Arrested development of embryonic red cell precursors in mouse embryos lacking transcription factor GATA-1. Proc. Natl. Acad. Sci. USA 1996;93:12355–12358. [PubMed: 8901585]

Gebuhr TC, Kovalev GI, Bultman S, Godfrey V, Su L, Magnuson T. The role of Brg1, a catalytic subunit of mammalian chromatin-remodeling complexes, in T cell development. J. Exp. Med 2003;198:1937–1949. [PubMed: 14676303]

Hu X, Eszterhas S, Pallazzi N, Bouhassira EE, Fields J, Tanabe O, Gerber SA, Bulger M, Engel JD, Groudine M, et al. Transcriptional interference among the murine beta-like globin genes. Blood 2007;109:2210–2216. [PubMed: 17077320]

Indra AK, Dupe V, Bornert JM, Messaddeq N, Yaniv M, Mark M, Chambon P, Metzger D. Temporally controlled targeted somatic mutagenesis in embryonic surface ectoderm and fetal epidermal keratinocytes unveils two distinct developmental functions of BRG1 in limb morphogenesis and skin barrier formation. Development 2005;132:4533–4544. [PubMed: 16192310]

Kadam S, McAlpine GS, Phelan ML, Kingston RE, Jones KA, Emerson BM. Functional selectivity of recombinant mammalian SWI/SNF subunits. Genes Dev 2000;14:2441–2451. [PubMed: 11018012]

Kingsley PD, Malik J, Fantauzzo KA, Palis J. Yolk sac-derived primitive erythroblasts enucleate during mammalian embryogenesis. Blood 2004;104:19–25. [PubMed: 15031208]

Kingsley PD, Malik J, Emerson RL, Bushnell TP, McGrath KE, Bloedorn LA, Bulger M, Palis J. “Maturational” globin switching in primary primitive erythroid cells. Blood 2006;107:1665–1672. [PubMed: 16263786]

Koni PA, Joshi SK, Temann UA, Olson D, Burkly L, Flavell RA. Conditional vascular cell adhesion molecule 1 deletion in mice: impaired lymphocyte migration to bone marrow. J. Exp. Med 2001;193:741–754. [PubMed: 11257140]

Kwon CS, Wagner D. Unwinding chromatin for development and growth: a few genes at a time. Trends Genet 2007;23:403–412. [PubMed: 17566593]

Leder A, Daugherty C, Whitney B, Leder P. Mouse zeta- and alpha-globin genes: embryonic survival, alpha-thalassemia, and genetic background effects. Blood 1997;90:1275–1282. [PubMed: 9242562]

Lee JS, Yu Q, Shin JT, Sebzda E, Bertozzi C, Chen M, Mericko P, Stadtfeld M, Zhou D, Cheng L, et al. Klf2 is an essential regulator of vascular hemodynamic forces in vivo. Dev. Cell 2006;11:845–857. [PubMed: 17141159]

Margueron R, Trojer P, Reinberg D. The key to development: interpreting the histone code? Curr. Opin. Genet. Dev 2005;15:163–176. [PubMed: 15797199]

Matsumoto S, Banine F, Struve J, Xing R, Adams C, Liu Y, Metzger D, Chambon P, Rao MS, Sherman LS. Brg1 is required for murine neural stem cell maintenance and gliogenesis. Dev. Biol 2006;289:372–383. [PubMed: 16330018]

McGrath KE, Palis J. Hematopoiesis in the yolk sac: more than meets the eye. Exp. Hematol 2005;33:1021–1028. [PubMed: 16140150]

McGrath KE, Koniski AD, Malik J, Palis J. Circulation is established in a stepwise pattern in the mammalian embryo. Blood 2003;101:1669–1676. [PubMed: 12406884]

Mikkola HK, Gekas C, Orkin SH, Dieterlen-Lievre F. Placenta as a site for hematopoietic stem cell development. Exp. Hematol 2005;33:1048–1054. [PubMed: 16140153]

Orkin SH, Zon LI. Genetics of erythropoiesis: induced mutations in mice and zebrafish. Annu. Rev. Genet 1997;31:33–60. [PubMed: 9442889]

Palis J, Robertson S, Kennedy M, Wall C, Keller G. Development of erythroid and myeloid progenitors in the yolk sac and embryo proper of the mouse. Development 1999;126:5073–5084. [PubMed: 10529424]

Peters LL, Shivdasani RA, Liu SC, Hanspal M, John KM, Gonzalez JM, Brugnara C, Gwynn B, Mohandas N, Alper SL, et al. Anion exchanger 1 (band 3) is required to prevent erythrocyte membrane surface loss but not to form the membrane skeleton. Cell 1996;86:917–927. [PubMed: 8808627]

Phelan ML, Sif S, Narlikar GJ, Kingston RE. Reconstitution of a core chromatin remodeling complex from SWI/SNF subunits. Mol. Cell 1999;3:247–253. [PubMed: 10078207]

Reyes JC, Barra J, Muchardt C, Camus A, Babinet C, Yaniv M. Altered control of cellular proliferation in the absence of mammalian brahma (SNF2alpha). EMBO J 1998;17:6979–6991. [PubMed: 9843504]

Sato, TN.; Loughna, S. Vasculogenesis and angiogenesis. In: Rossant, J.; Tam, PL., editors. Mouse Development: Patterning, Morphogenesis, and Organogenesis. Academic Press; San Diego: 2002. p. 211-233.

Schlaeger TM, Qin Y, Fujiwara Y, Magram J, Sato TN. Vascular endothelial cell lineage-specific promoter in transgenic mice. Development 1995;121:1089–1098. [PubMed: 7743922]

Schwarz DG, Griffin CT, Schneider EA, Yee D, Magnuson T. Genetic analysis of sorting nexins 1 and 2 reveals a redundant and essential function in mice. Mol. Biol. Cell 2002;13:3588–3600. [PubMed: 12388759]

Soriano P. Generalized lacZ expression with the ROSA26 Cre reporter strain. Nat. Genet 1999;21:70–71. [PubMed: 9916792]

Southgate CD, Chishti AH, Mitchell B, Yi SJ, Palek J. Targeted disruption of the murine erythroid band 3 gene results in spherocytosis and severe haemolytic anaemia despite a normal membrane skeleton. Nat. Genet 1996;14:227–230. [PubMed: 8841202]

Strobeck MW, Reisman DN, Gunawardena RW, Betz BL, Angus SP, Knudsen KE, Kowalik TF, Weissman BE, Knudsen ES. Compensation of BRG-1 function by Brm: insight into the role of the core SWI-SNF subunits in retinoblastoma tumor suppressor signaling. J. Biol. Chem 2002;277:4782–4789. [PubMed: 11719516]

Trimborn T, Gribnau J, Grosveld F, Fraser P. Mechanisms of developmental control of transcription in the murine alpha-and beta-globin loci. Genes Dev 1999;13:112–124. [PubMed: 9887104]

Tsai FY, Keller G, Kuo FC, Weiss M, Chen J, Rosenblatt M, Alt FW, Orkin SH. An early haematopoietic defect in mice lacking the transcription factor GATA-2. Nature 1994;371:221–226. [PubMed: 8078582]

Warren AJ, Colledge WH, Carlton MB, Evans MJ, Smith AJ, Rabbitts TH. The oncogenic cysteine-rich LIM domain protein rbtn2 is essential for erythroid development. Cell 1994;78:45–57. [PubMed: 8033210]

Wong PM, Chung SW, Reicheld SM, Chui DH. Hemoglobin switching during murine embryonic development: evidence for two populations of embryonic erythropoietic progenitor cells. Blood 1986;67:716–721. [PubMed: 3947744]

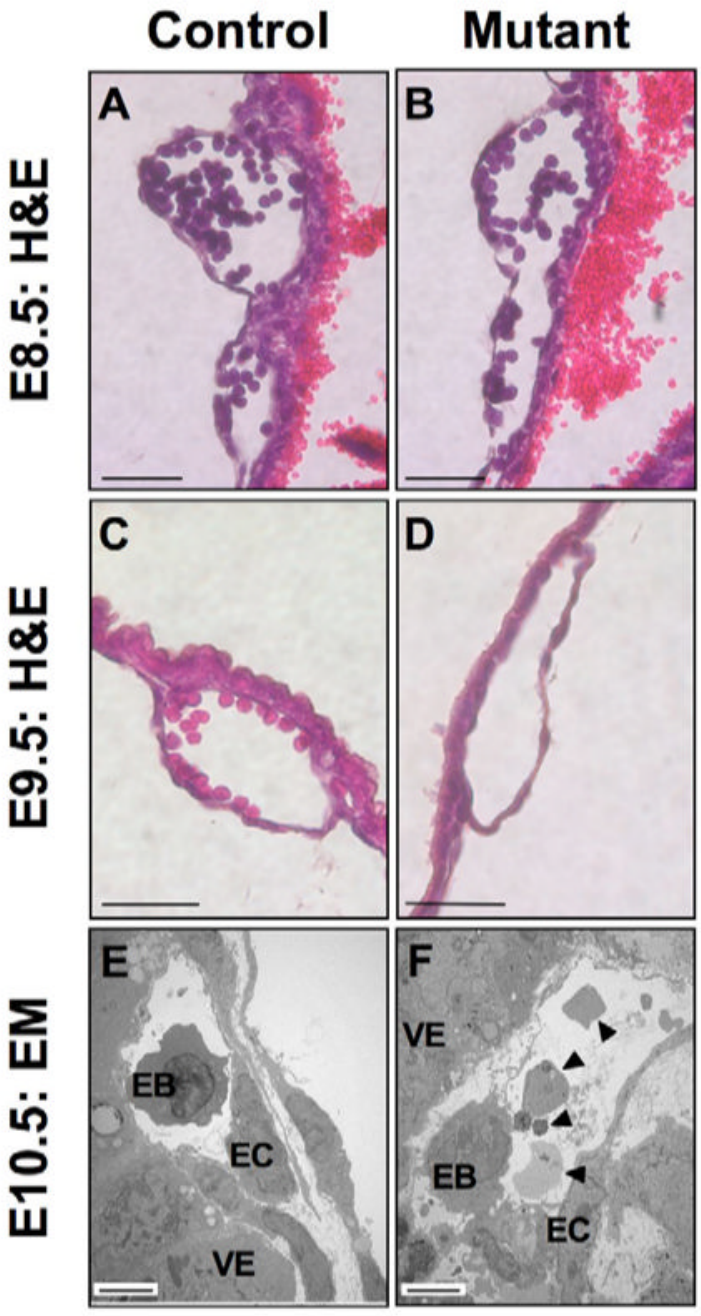

Fig. 1. Yolk sac-derived blood cells from *Brg1$^{fl/fl}$:Tie2-Cre$^{+/0}$* embryos are scarce and morphologically abnormal by E9.5

(**A,B**) Histological sections of *Brg1$^{fl/fl}$* (A) and *Brg1$^{fl/fl}$:Tie2-Cre$^{+/0}$* (B) yolk sac vessels filled with hematopoietic blood cell progenitors, from littermate E8.5 embryos. (**C,D**) Histological sections of a *Brg1$^{fl/fl}$* yolk sac vessel (C) and a *Brg1$^{fl/fl}$:Tie2-Cre$^{+/0}$* yolk sac vessel (D). The mutant vessel is devoid of embryonic blood cells. Scale bars in A-D: 40 μm. (**E,F**) Transmission electron micrographs of E10.5 *Brg1$^{fl/+}$* (E) and *Brg1$^{fl/fl}$:Tie2-Cre$^{+/0}$* (F) yolk sac blood vessels. Arrowheads indicate abnormal embryonic blood cells and blood cell fragments. EB, embryonic blood cell; EC, endothelial cell; VE, visceral endoderm. Scale bars in E,F: 5 μm.

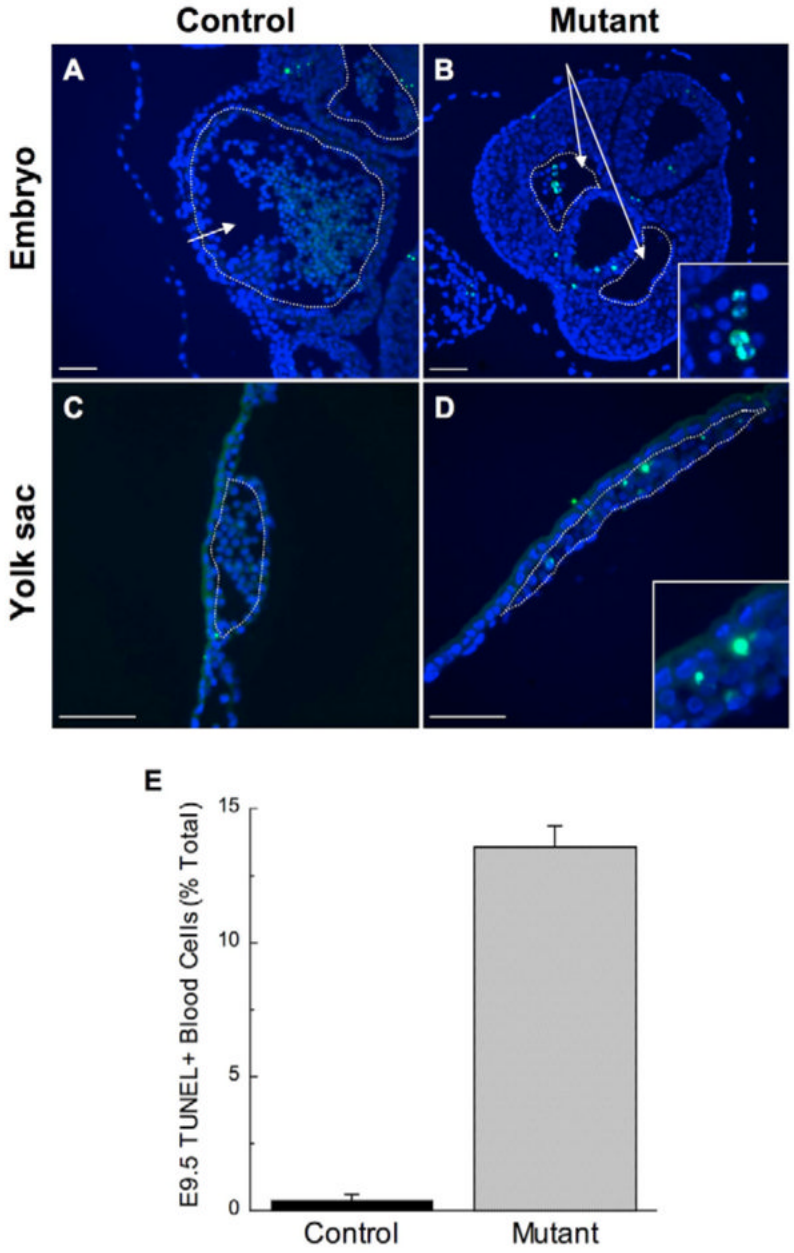

Fig. 2. Yolk sac-derived blood cells from *Brg1$^{fl/fl}$:Tie2-Cre$^{+/0}$* embryos undergo apoptosis at E9.5

(**A-D**) TUNEL staining on histological sections of E9.5 littermate control *Brg1$^{fl/fl}$* (A) and mutant *Brg1$^{fl/fl}$:Tie2-Cre$^{+/0}$* (B) embryos, and their corresponding control (C) and mutant (D) yolk sacs. The images were merged from separate DAPI (blue) and TUNEL (green) acquisitions. The arrow in A points to the lumen of an embryonic heart (outlined in white), filled with embryonic blood cells. The inset in B focuses on TUNEL-positive blood cells found within one of the paired dorsal aortae (arrows and outlined in white) of the mutant embryo. No TUNEL-positive blood cells are detected in the control yolk sac vessel (C; outlined in white), but TUNEL-positive blood cells are evident in the mutant yolk sac vessel (D, outlined in white; shown at higher magnification in the inset in D). Scale bars: 100 μm. (**E**) Mean percentages of TUNEL-positive blood cells from multiple serial sections of two control and two mutant

embryos stained in three independent experiments. A total of 596 and 362 blood cells were counted from control and mutant sections, respectively. Errors were calculated as s.e.m.

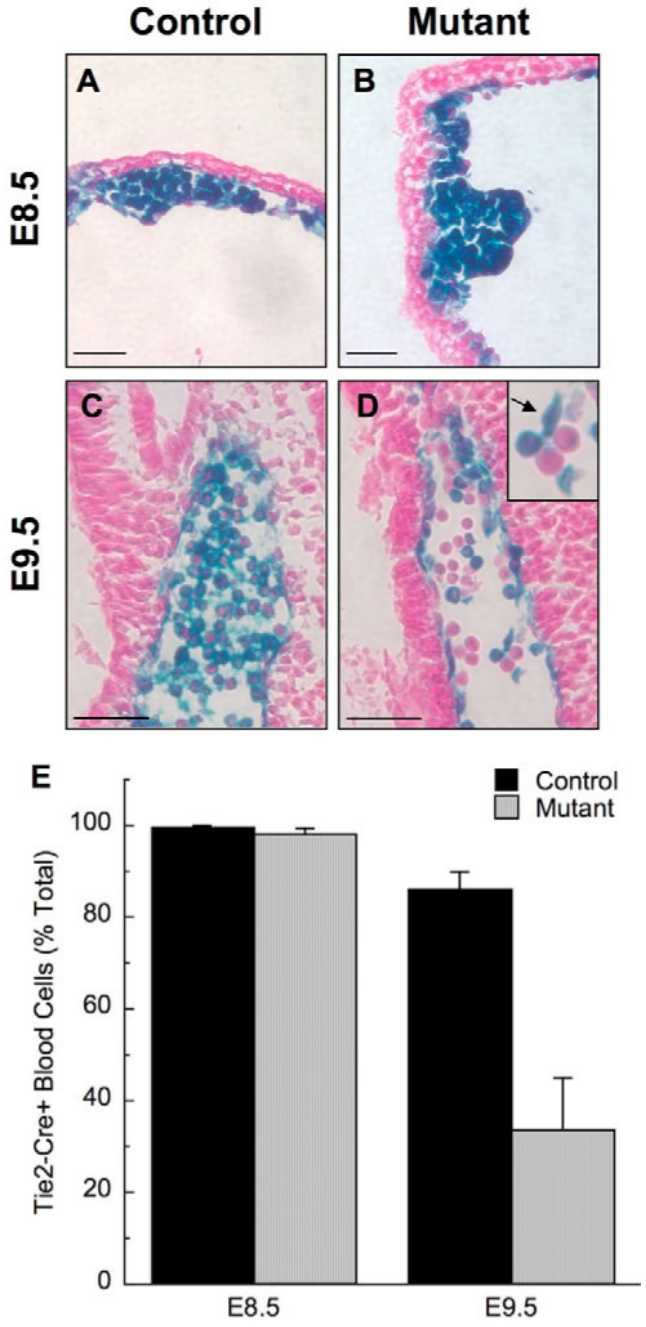

Fig. 3. *Brg1$^{fl/fl}$:Tie2-Cre$^{+/0}$* blood cells are largely depleted by E9.5

Mutant embryos were crossed onto the ROSA26 reporter line and were stained with X-gal for detection of β-galactosidase, which marks Cre-mediated excision events. (**A,B**) Blood vessels from *Brg1$^{fl/+}$;R26R$^{R/+}$:Tie2-Cre$^{+/0}$* control (A) and *Brg1$^{fl/fl}$;R26R$^{R/+}$:Tie2-Cre$^{+/0}$* mutant (B) E8.5 yolk sacs contain a comparable number of blood cell precursors, the majority of which express *Tie2-Cre*. (**C**) The majority of circulating blood cell precursors still express *Tie2-Cre* at E9.5 in *Brg1$^{fl/+}$;R26R$^{R/+}$:Tie2-Cre$^{+/0}$* control embryos. (**D**) By contrast, fewer circulating blood cell precursors are found in *Brg1$^{fl/fl}$;R26R$^{R/+}$:Tie2-Cre$^{+/0}$* mutant embryos at E9.5, but, of those cells that persist, the proportion of cells that express *Tie2-Cre* and are presumably deficient for *Brg1* expression is dramatically decreased when compared with the

blood cells in the control embryo (C). The arrow in the inset points to an abnormally shaped mutant (*Brg1*$^{fl/fl}$*:Tie2-Cre*$^{+/0}$) blood cell. Scale bar: 40 μm. (**E**) Mean percentages of *Tie2-Cre*-positive (β-galactosidase-positive) blood cells from multiple serial sections of two control and two mutant embryos at E8.5, and two control and two mutant embryos at E9.5, carrying the ROSA26 reporter, as detected by X-gal staining. A total of 941 and 487 blood cells were counted from control and mutant sections at E8.5, respectively, while a total of 1,656 and 893 blood cells were counted from control and mutant sections at E9.5, respectively. Errors were calculated as s.e.m.

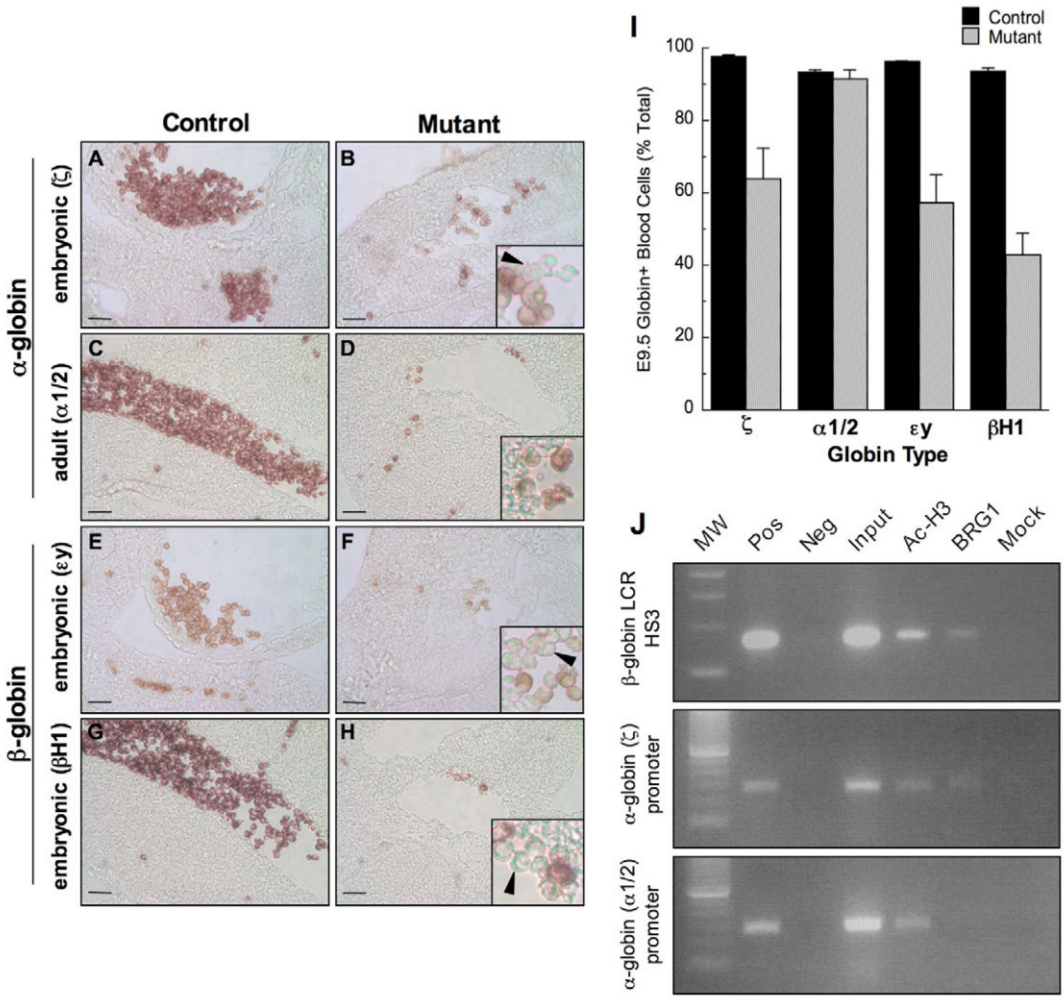

Fig. 4. ***Brg1$^{fl/fl}$:Tie2-Cre$^{+/0}$*** **primitive erythroblasts have embryonic globin deficits**

(**A-H**) Cryosections from an E9.5 control *Brg1$^{fl/fl}$* embryo (A,C,E,G) and a mutant *Brg1$^{fl/fl}$:Tie2-Cre$^{+/0}$* embryo (B,D,F,H) were subjected to in situ hybridization with probes against embryonic and adult α-globins and embryonic β-globins. Almost all embryonic blood cells from the control embryo express embryonic *α*-globin *ζ*(A), adult *α1/2*-globins (C), embryonic β-globin *ϵy* (E) and embryonic *βH1*-globin (G). In the mutant embryo, adult *α1/2*-globin expression is normal (D), but many embryonic blood cells can be detected that express little or no embryonic *ζ* (B), *ϵy* (F) or *βH1* (H), as indicated by the arrowheads in the respective insets. Scale bars: 40 μm. (**I**) Mean percentages of globin-expressing blood cells from multiple serial sections of two control and two mutant embryos at E9.5, as detected by in situ hybridization in three independent experiments. Total blood cells counted from control/mutant sections with each probe were: *ζ*, 1521/907; *α1/2*, 1292/635; *ϵy*; 977/721; and *βH1*, 492/818. Errors were calculated as s.e.m. (**J**) ChIP assay demonstrating that BRG1 is recruited to the β-globin LCR DNAse I hypersensitive site 3 (HS3) and the *ζ* promoter in primitive erythrocytes. No evidence of BRG1 recruitment is detected at the *α1/2* promoter. MW, 100 bp molecular weight standard; Pos, wild-type genomic DNA served as a positive control for the PCR; Neg, no DNA was amplified as a negative control; Input, total chromatin, sheared but not immunoprecipitated; Ac-H3, ChIP material immunoprecipitated with an antibody against pan-acetyl histone 3 (H3), a mark of an open chromatin structure; BRG1, ChIP material immunoprecipitated with an antibody against BRG1; Mock, mock immunoprecipitation in

which the sample was not treated with antibody but was otherwise handled identically to the ChIP samples.

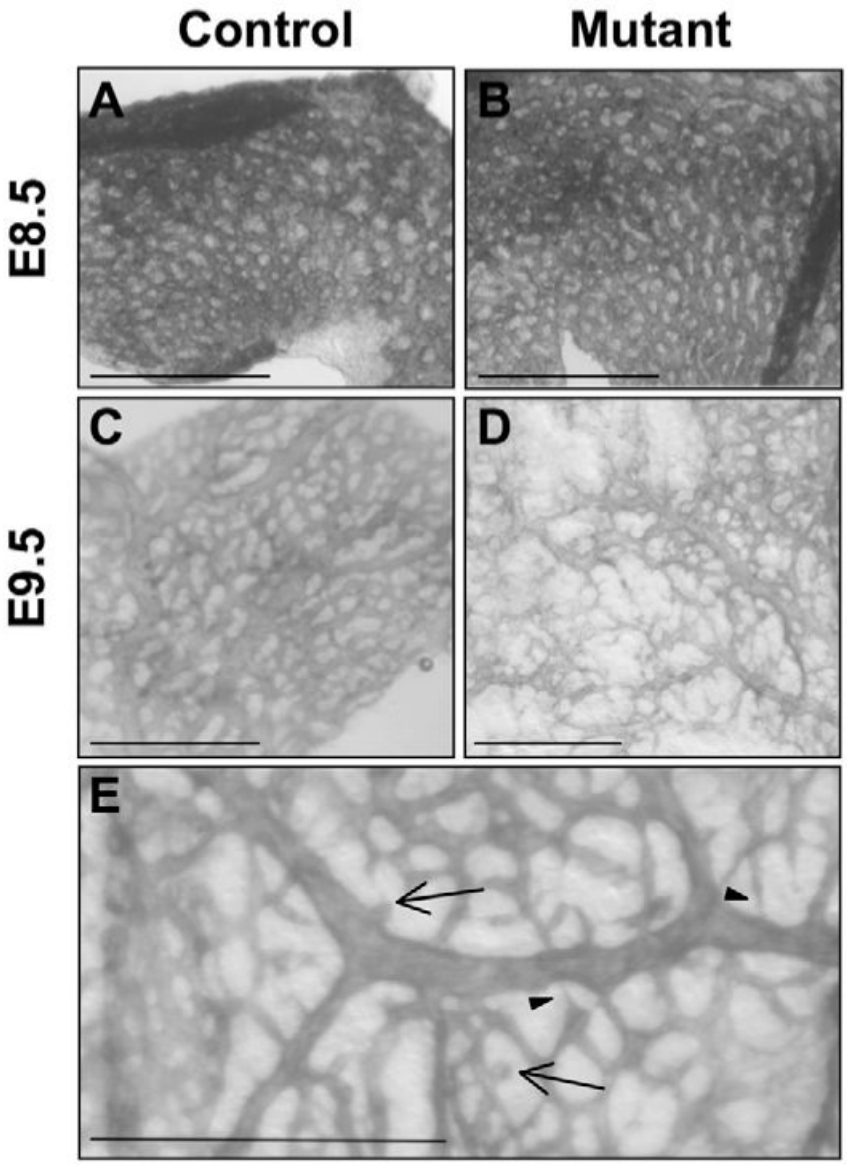

Fig. 5. *Brg1$^{fl/fl}$:Tie2-Cre$^{+/0}$* yolk sac vascular remodeling is abnormal

(**A,B**) Anti-PECAM1-stained yolk sacs from E8.5 littermate *Brg1$^{fl/+}$* (A) and *Brg1$^{fl/fl}$:Tie2-Cre$^{+/0}$* (B) embryos display a comparable vascular plexus. (**C,D**) Anti-PECAM1-stained yolk sacs from E9.5 littermate *Brg1$^{fl/fl}$* (C) and *Brg1$^{fl/fl}$:Tie2-Cre$^{+/0}$* (D) embryos. (**E**) A higher magnification view of a different region of the *Brg1$^{fl/fl}$:Tie2-Cre$^{+/0}$* yolk sac shown in D. Arrows indicate sprouting or regressing vessels; arrowheads indicate abnormally thin vessels. Scale bars: 500 μm.

Table 1

Brg1$^{fl/fl}$:Tie2-Cre$^{+/0}$ embryos die during development

Genotype	Live progeny (expected progeny)
Brg1$^{fl/+}$	52 (38.75)
Brg1$^{fl/+}$;Tie2-Cre$^{+/0}$	46 (38.75)
Brg1$^{fl/fl}$	57 (38.75)
Brg1$^{fl/fl}$;Tie2-Cre$^{+/0}$	0 (38.75)

Brg1$^{fl/fl}$ females were mated with *Brg1$^{fl/+}$:Tie2-Cre$^{+/0}$* males, and live progeny from 24 litters were genotyped and scored at weaning. No live *Brg1$^{fl/fl}$:Tie2-Cre$^{+/0}$* mice were recovered [$\chi^2(3_{dof})$: $P<0.001$].

Table 2

Brm$^{-/-}$;*Brg1*$^{fl/+}$:*Tie2-Cre*$^{+/0}$ embryos do not die during development

Genotype	Live progeny (expected progeny)
Brm$^{-/-}$;*Brg1*$^{fl/+}$	26 (18)
Brm$^{-/-}$;*Brg1*$^{fl/+}$;*Tie2-Cre*$^{+/0}$	24 (18)
Brm$^{-/-}$;*Brg1*$^{fl/fl}$	21 (18)
Brm$^{-/-}$;*Brg1*$^{fl/fl}$;*Tie2-Cre*$^{+/0}$	1 (18)

Brm$^{-/-}$;*Brg1*$^{fl/fl}$ females were mated with *Brm*$^{-/-}$;*Brg1*$^{fl/+}$:*Tie2-Cre*$^{+/0}$ males, and live progeny from 12 litters were genotyped and scored at weaning. Only one live *Brm*$^{-/-}$;*Brg1*$^{fl/fl}$:*Tie2-Cre*$^{+/0}$ mouse was recovered [$\chi^2(3_{dof})$: $P<0.001$].