FGFs, Wnts and BMPs mediate induction of VEGFR-2 (*Quek-1*) expression during avian somite development

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Abstract

Regulation of VEGFR-2 (Quek1) is an important mechanism during blood vessel formation. In the paraxial mesoderm, *Quek1* expression is restricted to the lateral portion of the somite and later to sclerotomal cells surrounding the neural tube. By implanting FGF 8b/8c or SU 5402 beads into the paraxial mesoderm, we show that FGF8 in addition to BMP4 from the intermediate mesoderm (IM) is a positive regulator of VEGFR-2 (*Quek1*) expression in the quail embryo. The expression of *Quek1* in the medial somite half is normally repressed by the notochord and Sfrps-expression in the neural tube. Over-expression of Wnt 1/3a also results in an up-regulation of *Quek1* expression in the somites. We also show that up-regulation of FGF8/Wnt 1/3a leads to an increase in the number of endothelial cells, whereas inhibition of FGF and Wnt signaling by SU 5402 and Sfrp-2 results in a loss of endothelial cells. Our results demonstrate that the regulation of *Quek1* expression in the somites is mediated by the cooperative actions of BMP4, FGF8 and Wnt-signaling pathways.
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Introduction

Blood vessels in the early embryo are formed by the proliferation and differentiation of endothelial precursors, the angioblasts, in a process called vasculogenesis. Angiogenesis is the growth and sprouting of new vessels from existing ones. The growth and maintenance of the blood and lymphatic vascular systems is to a large extent mediated by members of the vascular endothelial growth factor (VEGF) family via their tyrosine kinase receptors (VEGFRs) that are expressed in angioblastic and endothelial cells (Ferrara, 2000). VEGF supports proliferation and survival of endothelial cells and induces the expression of antiapoptotic proteins in endothelial cells (Alon et al., 1995; Benjamin et al., 1999). Endothelial tyrosine kinase receptors are of fundamental importance for the transmission of both differentiation and angiogenic signals from the environment to the endothelium (Wilting et al., 2003). VEGFs bind with high affinity to five receptors: three receptor tyrosine kinases called VEGFR-1 (Flt-1), VEGFR-2 (Flk-1/KDR) and VEGFR-3 (Flt-4), as well as two non-kinase receptors, neuropilin-1 (NRP-1) and NRP-2.

In the quail, the homologue of VEGFR-2 has been cloned and named Quek1 (Eichmann et al., 1993, 1997). Quek1 possesses 69% and 71% identity to murine Flk-1 and human KDR. VEGFR-2 expressing cells isolated from chick blastoderm and cultured *in vivo* can give rise to both endothelial and hematopoietic cells (Eichmann et al., 1997). VEGFR-2 deficient mice failed to form yolk-sac blood islands and lacked organized blood vessel formation in the embryo proper (Shalaby et al., 1995). Thus, embryonic blood vessel formation depends on this receptor of which the expression has been studied in detail (Yamaguchi et al., 1993; Quinn et al., 1993; Eichmann et al., 1993; Wilting et al., 1997; Nimmagadda et al., 2004). In the quail paraxial mesoderm, expression of *Quek1* can be observed in the lateral portion of both the segmental plate mesoderm and the epithelial somite. Initial expression in the somite is restricted to the dorsolateral quadrant (Eichmann et al., 1993; Nimmagadda et al., 2004; Wilting et al., 1997). Later, a medial expression domain is established in the sclerotome adjacent to the neural tube. In the avascular notochord, *Quek1* is

being expressed from day 4 onwards (Eichmann et al., 1993; Nimmagadda et al., 2004; Wilting et al., 1997). The mechanisms controlling VEGFR-2 expression can give us a key to understand the regulation of these expression patterns.

The lateral plate is subdivided into somatic and splanchnic mesoderm by the coelomic cavity. By means of quail/chick transplantation experiments, it has been shown that both somitic and splanchnic mesoderm have the potential to give rise to endothelial progenitors (Wilting et al., 1997; Pardanaud and Dieterlen-Lievre, 1999; Pardanaud et al., 1987). Since angioblasts do not cross the embryonic midline (Klessinger and Christ, 1996), it has been suggested that notochord-derived signals inhibit midline crossing and negatively regulate blood vessel formation. In contrast, 3 to 5 days after grafting quail segmental plate to chick, QH1$^+$ vascular plexuses were found around and inside the neural tube. Further some QH$^+$ cells had crossed the midline either as single cells or organized into vascular structures (Pouget et al., 2006). Our previous observations demonstrated that the BMP4 signaling pathway is involved in the regulation of *Quek1* expression (Nimmagadda et al., 2005). FGF8 is found to be expressed in the intermediate mesoderm along with BMP4. Further, FGF and BMP signaling have been found to induce cardiac differentiation (Lough et al., 1996; Alsan and Schultheiss, 2002). Fibroblast growth factors (FGFs) induce proliferation and differentiation of epithelial and mesenchymal cells. Several FGFs have previously been found to be produced by tumor cells and induce angiogenesis (MacArthur et al., 1995; Tanaka et al., 1995; Johnson et al., 1998; Marsh et al., 1999; Dorkin et al., 1999; Gerwins et al., 2000). Several Wnt receptors and transcriptional effectors are expressed in human and mouse endothelial cells and also in many tumor types. Wnt/β-catenin signaling has been shown to promote endothelial cell proliferation whereas suppression of Wnt activity reduces Flk$^+$ embryonic stem cells (Masckauchan et al., 2005; Wang et al., 2006).

In the present study, we extended our previous work (Nimmagadda et al., 2005) on the regulation of *Quek1* expression in the somite. We found that apart from the inhibiting effect of the notochord and the inducing role of BMP4 on *Quek1* expression, additional signaling mechanisms are involved in this process. We show that FGF8 from the intermediate mesoderm and Wnts (1 and 3a) from the neural tube are involved in the induction of *Quek1* expression. We also show that over-expression of FGF8 or Wnt-1 or 3a induces the formation of additional endothelial cells whereas SU 5402 and Sfrp-2 inhibits *Quek1* expression leading to a loss of endothelial cells. These results demonstrate that the regulation of *Quek1* expression in somites is mediated by cooperative actions of BMP, FGF and Wnt-signaling pathways.

Materials and methods

Preparation of quail embryos

Fertilized quail eggs (*Coturnix coturnix japonica*) were incubated at 38 °C under 80% humidity and the embryos were staged according to Hamburger and Hamilton (1952). Experiments were performed on embryos at stages 12–14.

Implantation of beads

(a) Purified recombinant mouse FGF-8b and 8c protein (R&D Systems) were diluted in PBS to a concentration of 1 μg/μl. For application of FGF, Affigel beads of approximately 80–120 μm in diameter (BioRad Laboratories) were rinsed in PBS and incubated with FGF-8b or 8c protein solution for overnight at 4 °C. Beads soaked in PBS were used as controls.

(b) FGF signaling inhibitor SU 5402 (Calbiochem) was dissolved in dimethyl sulfoxide to a concentration of 10 mM. For application of SU 5402, AG 1-X2 Resin carrier beads (diameter: 100 μm; BioRad Laboratories) were incubated for overnight in SU 5402 solution. Control beads were incubated in dimethyl sulfoxide.

For bead implantation, paraxial mesoderm (somite 14–19 of HH-stage 12–13 embryo) was punctured with an electrolytically sharpened tungsten needle, and a bead was inserted into the mesenchyme using a blunt glass needle. Embryos were reincubated for 16–20 h, processed for whole mount *in situ* hybridization. None of the controls had an effect on *Quek1* expression.

Cell injection

Wnt3a-, Wnt1-, Wnt4- and Sfrp2-expressing cells were a gift from Andreas Kispert (Medizinische Hochschule Hannover, Germany). Cell lines were cultured as described elsewhere (Lamb et al., 1993). Confluent cultures were harvested, cells were washed in phosphate-buffered saline (PBS), pelleted and resuspended in a minimal volume of medium. For cell injection, the ectoderm (at the level of somite I–V of HH stage 13–14 embryos) was punctured with a tungsten needle. With the help of a blunt glass needle, a tunnel was made below the ectoderm and concentrated cell suspensions were locally applied with a micropipette along the length of the tunnel. Embryos were reincubated from 16 to 20 h, processed for whole-mount *in situ* hybridization. Control cells showed no effect on target genes expression (not shown).

In situ hybridization

Embryos were fixed overnight at 4 °C in 4% PFA. Embryos were washed twice in PBT, dehydrated in methanol and stored at 4 °C. Whole mount *in situ* hybridization was performed as previously described (Nieto et al., 1996). Selected stained embryos were embedded in 4% agar and sectioned with a Leica Vibratome at 50 μm. For Quek1, we used the cloned Quek1 4500-bp fragment as template. Linearization was performed with *Hin*dIII and *Sph*I (Quek1/VEGFR-2) to produce antisense and sense probes.

Immunohistochemistry on whole mounts for the detection of quail endothelial cells

Selected embryos after *in situ* hybridization were used for immunohistochemistry, fixed overnight in 4% paraformaldehyde (PFA), washed in PBS. Following a brief wash in PBS, embryos were sectioned, incubated overnight with monoclonal QH1 antibody (DSHB; 1:5 in PBS). After extensive washing in PBS, embryos were incubated overnight in secondary antibody (Cy3-conjugated goat anti-mouse IgG antibody; Jackson ImmunoResearch, 1:100 in PBS). Subsequently, sections were washed in PBS, mounted in Mowiol (Merk) and analyzed with an epifluorescence microscope (Axiophot; Zeiss).

Results

FGF8 signals from the intermediate mesoderm (IM) induce Quek1 expression in the somite

Expression of *Quek1* is restricted to the lateral portion of the somite (Figs. 1A–B). Previous studies have shown that *BMP4* expressed in the IM and/or LPM is required for induction of
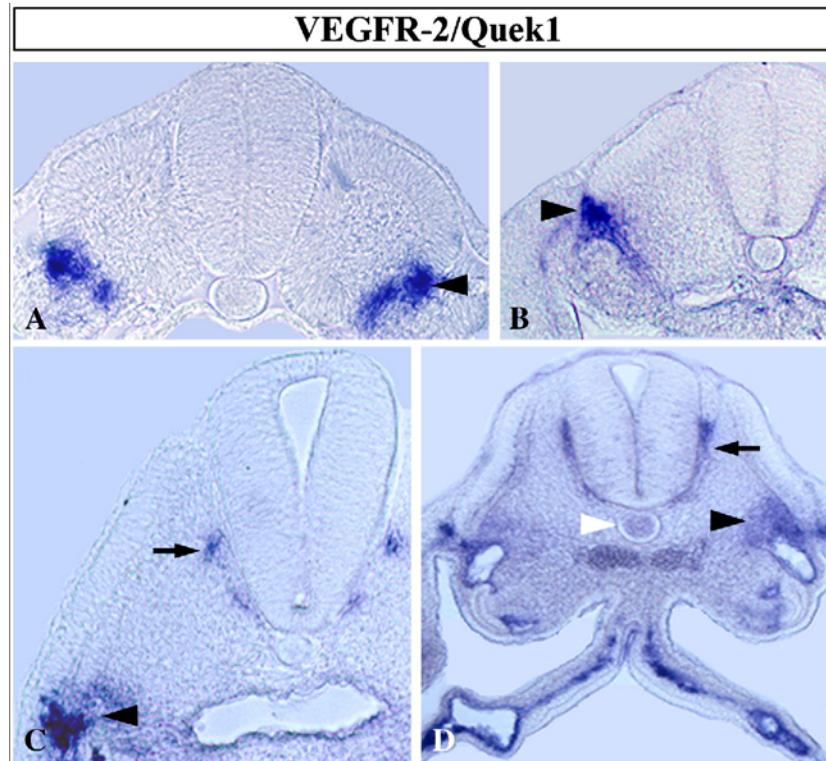
Fig. 1. Expression pattern of VEGFR-2 (*Quek1*) during quail somite development. (A) Epithelial somite of HH stage 13 quail embryo, *Quek1* expression found in the lateral portion of somite and in the intermediate mesoderm (*arrowhead*). (B) The somite has compartmentalized and expression of *Quek1* is observed in the lateral portion of both the dermomyotome and sclerotome (*arrowhead*) of HH-stage 13 quail embryo. (C) Transverse section of a matured somite of an HH-stage 15 quail embryo showing the initiation of *Quek1* expression in the sclerotome, surrounding the neural tube (*arrow*); expression in the somite and the intermediate mesoderm remains the same (*arrowhead*). (D) Somite region of HH-stage 23 quail embryo, *Quek1* expression in the sclerotome surrounding the neural tube has become much stronger (*arrow*) than in earlier stages, in the notochord (*white arrowhead*) and in the lateral portion of the somite (*arrowhead*).

Quek1 expression and blood vessel formation in the somites (Nimmagadda et al., 2005). Our previous observations prompted us to look for additional signals that can induce *Quek1* expression in the somite. FGF8 expression is seen (data not shown) in the IM as reported earlier (Stolte et al., 2002). To test if FGF8 could be an additional signal inducing *Quek1* expression, we implanted beads soaked with FGF-8b or 8c protein into the paraxial mesoderm. After reincubation for 16–20 h, we found a strong up-regulation of *Quek1* expression ($n=11$) in the operated side (Figs. 2A–B, data not shown for FGF-8c as it was identical to the effect seen after FGF-8b over-expression). In addition, the number of endothelial cells around the beads was drastically increased (Figs. 2C–D, data not shown for FGF-8c over-expression) compared to the control side, when stained with QH1 antibody.

To confirm these results, we used SU 5402-soaked beads (Mohammadi et al., 1997), an inhibitor of FGF signaling, to determine whether blocking FGF signaling would have any effect on *Quek1* expression. Implantation of beads soaked with SU 5402 into the paraxial mesoderm results in a complete loss of *Quek1* expression ($n=9$) around the beads (Figs. 2E–F) in the operated side compared to the normal expression in the control side. Sections stained with QH1 shows a loss of endothelial cells in the operated side (Fig. 2G). This depicts that inhibition of FGF negatively regulates *Quek1* expression

in the somites, showing that FGF8 like BMP4 (Nimmagadda et al., 2005), promotes somitic *Quek1* expression.

Wnt signaling from the neural tube induces Quek1 expression

From HH-stage 15 onwards, *Quek1* is expressed in sclerotome cells surrounding the neural tube (Nimmagadda et al., 2004), where the expression becomes stronger during later stages (Figs. 1C–D). We showed earlier that this is based on an antagonistic role of BMP4 and noggin (Nimmagadda et al., 2005). We hypothesized that Wnts (Wnt-1, 3a and 4) expressed in the neural tube (Munsterberg et al., 1995; Marcelle et al., 1997) might also be involved in the process of *Quek1* induction, in association with BMP4. After injection of Wnt1- or Wnt3a-expressing cells into the paraxial mesoderm, *Quek1* expression is up-regulated ($n=9$) around the site of injection (Figs. 3A–B, data not shown for Wnt 3a which remains identical to the effect seen after Wnt1 over-expression) whereas, over-expression of Wnt-4 by injecting Wnt4-expressing cells does not have any effect on *Quek1* expression (data not shown). Sections stained with QH1 show an increased number of endothelial cells (Figs. 3C–D, data not shown for Wnt3a over-expression), comparable with the region where up-regulation of *Quek1* expression did occur (Fig. 3B). At the site of injection, the somites appear smaller in size when compared to the control side. It is a known
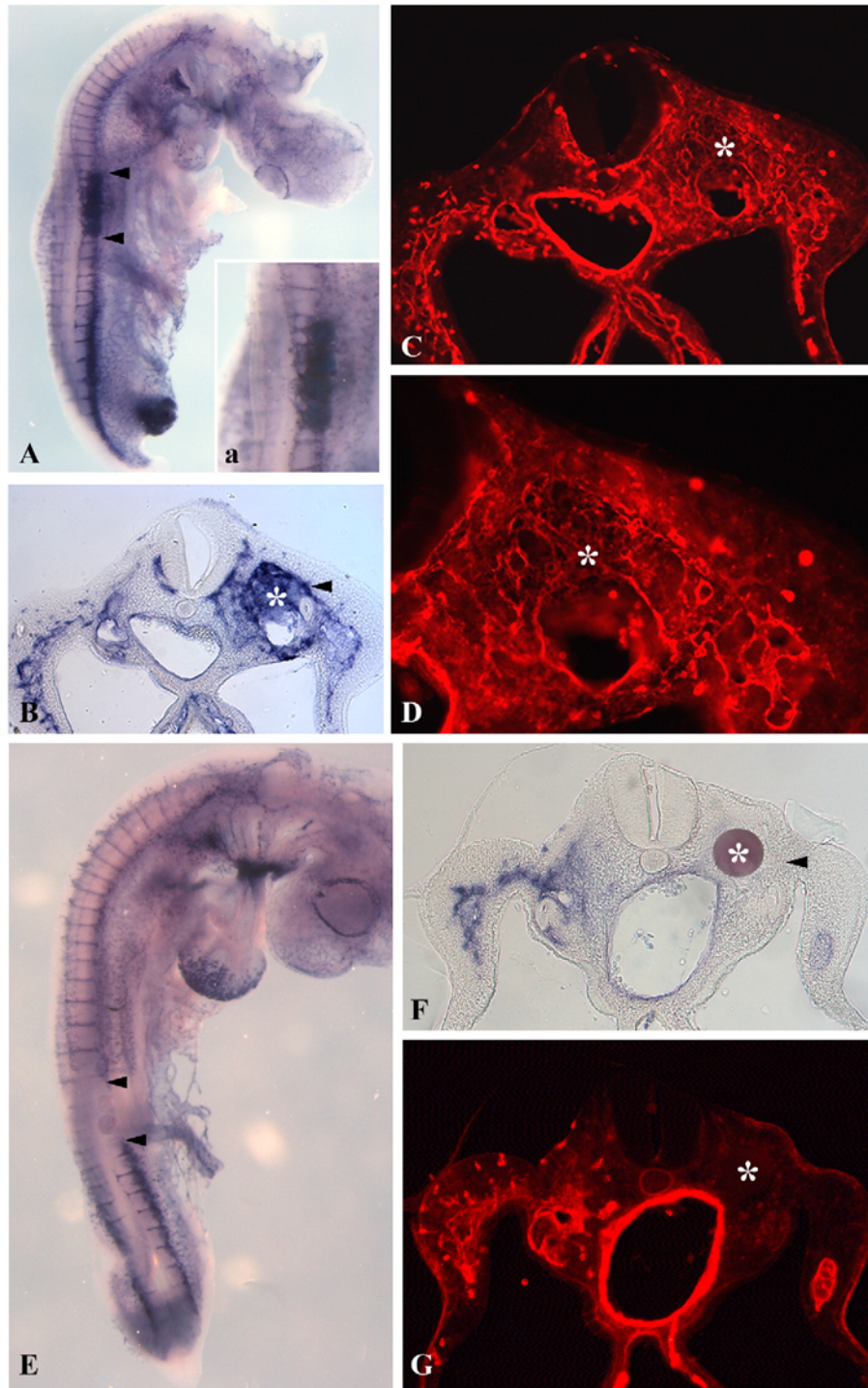
Fig. 2. (A–D) FGF8b induces expression of *Quek1* in the somite: (A) Implantation of beads coated with FGF8b protein, induction of *Quek1* expression (between *arrowheads*) in the operated side of the embryo. (a) Magnified image of the operated region of the embryo shown in panel A. (B) Transverse section through the operated region of the embryo in panel A, up-regulation of *Quek1* expression (*arrowhead*) around the bead (*asterisk*). (C) Section in panel B, stained with QH1 antibody, increased number of endothelial cells in the operated side of the somite around the bead comparable with *Quek1* induction region in panel B. (D) Enlarged view of operated side of the section in panel C, extensive increase in the endothelial cells around the bead. Position of bead is marked by *asterisks*. (E–G) Inhibitor of FGF signaling, SU 5402 proves the involvement of FGF signaling in *Quek1* induction: (E) Implantation of beads coated with SU 5402 into the somites, complete loss of *Quek1* expression (between *arrowheads*) is observed. (F) Transverse section through the operated region in panel A, loss of *Quek1* expression (*arrowhead*) correlates with the absence of endothelial cells (marked by QH1 antibody) in the operated side of section in panel G.

fact that over-expression of Wnts induces epithelialization leading to the formation of smaller somites as observed by us and others (Wagner et al., 2000; Schmidt et al., 2004; Linker et al., 2005; Geetha-Loganathan et al., 2006). Further, injection of Sfrp2-expressing cells into the paraxial mesoderm results in the down-regulation (*n* = 7) of *Quek1* expression (Figs. 3E–F). The
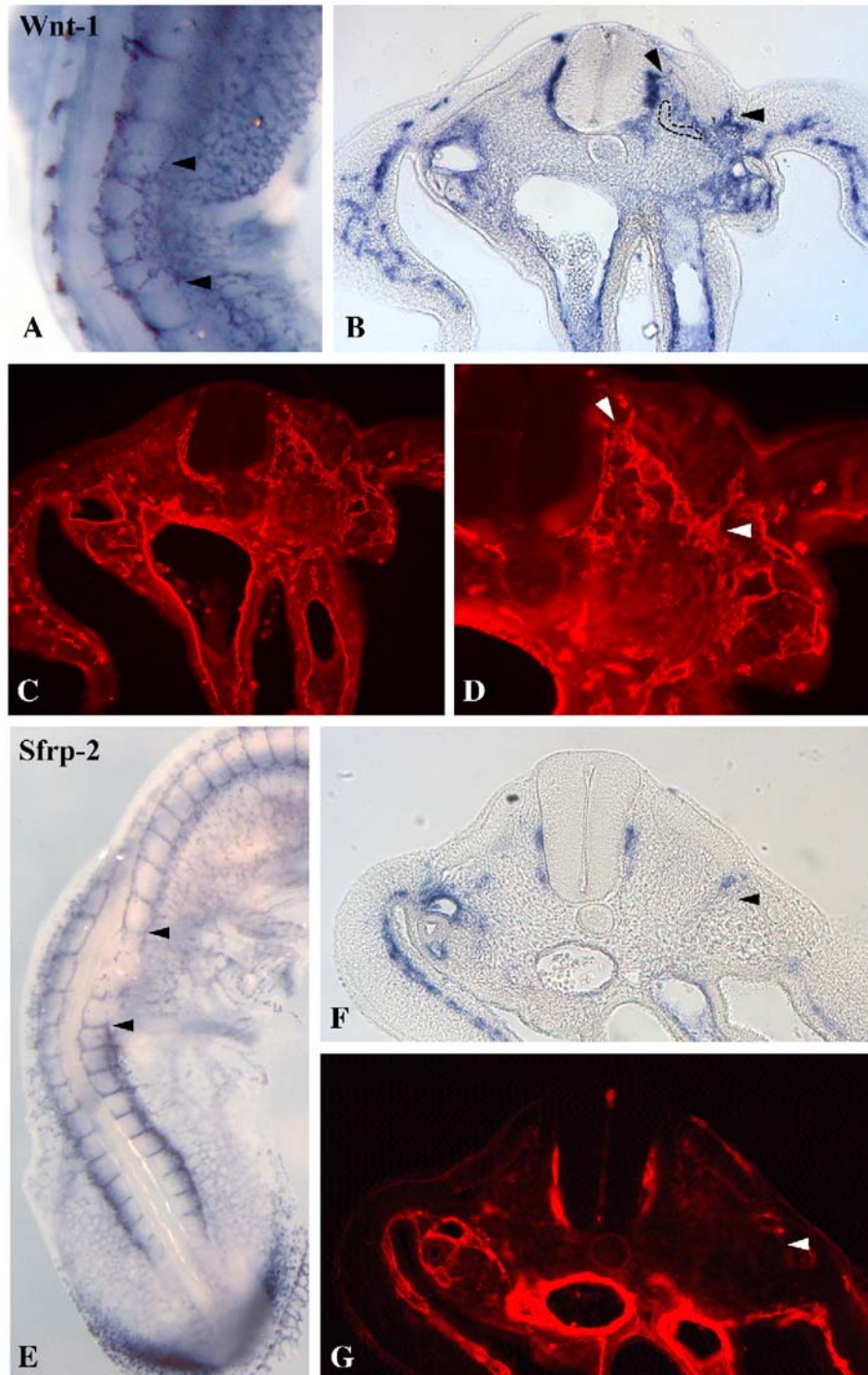
Fig. 3. (A–D) Wnt1 signaling up-regulates *Quek1* expression in the somites: (A) Injection of Wnt1-expressing cells into the epithelial somites leads to the induction of *Quek1* expression in the somites (between *arrowheads*), note the smaller somites at the region of cell injection. (B) Transverse section at the region of operation in panel A showing up-regulation of *Quek1* expression (*arrowheads*), position of cells is indicated by broken lines. (C) Same section in panel B counter stained with QH1 antibody, additional endothelial network formation seen, more clearly in the enlarged view (D) of the operated side (*arrowheads*). (E–G) Inhibition of Wnts by Sfrp-2 down-regulates *Quek1* expression: (E) Over-expression of Sfrp-2 results in the inhibition of *Quek1* expression (between *arrowheads*). (F) Section through the level of operation, inhibition of *Quek1* expression (*arrowhead*) correlates with the loss of endothelial cells (G) in the operated side (*arrowhead*).

down-regulation of *Quek1* expression corresponds with a reduced number of endothelial cells in the section (Fig. 3G) through the operated region. These data suggests that Wnt-1 and 3a from the neural tube induce *Quek1* expression.

Discussion

Quek1 (VEGFR-2) is a tyrosine-kinase receptor which is the quail homologue of KDR and flk-receptors found in human and

mouse (De Vries et al., 1992; Millauer et al., 1993). VEGFR-2 is expressed in endothelial cells of all types of blood vessels as well as lymphatic vessels. It is observed at very early stages of development in angioblasts, which makes it a key marker for endothelial cells and their precursors (Eichmann et al., 1993; Yamaguchi et al., 1993). It is thus important to study the potential roles played by various signaling molecules in the regulation of Quek1 (VEGFR-2). In the paraxial mesoderm, Quek1 expression (Figs. 1A–B) is normally restricted to its lateral portion (Eichmann et al., 1993; Nimmagadda et al., 2004; Wilting et al., 1997). Since the early somite is still plastic with respect to compartmentalization (Christ et al., 1992; Ordahl and Le Douarin, 1992; Sudo et al., 2001), it can be assumed that the restricted expression of Quek1 to the lateral portion of the somite is induced by signals from surrounding tissues. Accordingly, we found in a previous study that the signals responsible for Quek1 expression in somites are from the IM and/or LPM. We also found that BMP4 from the IM and/or LPM not only induces Quek1 expression but is also involved in vascular patterning (Nimmagadda et al., 2005).

We hypothesized that additional signaling mechanisms might be active in regulating Quek1 expression. A candidate signaling molecule which is expressed in the IM is FGF8 (own data (not shown); Stolte et al., 2002). From 23 FGF known family members, FGF-1, 2, 3 and 4 have previously been found to be produced by tumor cells and induce angiogenesis in vitro (reviewed in Bouck et al., 1996). Abnormal vascularization has also previously been found in tumors produced by FGF-transfected NBT-II cells (Jouanneau et al., 1995; Maniotis et al., 1999; Chang et al., 2000). Accordingly, we found an up-regulation of Quek1 expression in the somites after implantation of beads coated with FGF 8b or 8c protein. Tumors produced by FGF-8a and 8b-over-expressing cells were shown to have angiogenic morphology with a great number of large dilated vessels (MacArthur et al., 1995; Ghosh et al., 1996; Mattila et al., 2001). Our results show that FGF 8b or 8c not only induces Quek1 expression but are also involved in the induction of endothelial cell formation and hence in vascular patterning. In support of our results, recombinant FGF-8b protein was found to stimulate proliferation, migration and sprouting of immortomous brain capillary endothelial cells (IBECs) in vitro. The angiogenic potential of rFGF-8b was also reported in vivo in the chorioallantoic membrane (CAM) assay (Mattila et al., 2001). Besides VEGF, which has been proved to correlate with increased angiogenesis (Guidi et al., 1997) and poor prognosis of breast cancer (Toi et al., 1994; Gasparini et al., 1997; Relf et al., 1997), a high level of FGF-8 expression has been found in breast cancer (Marsh et al., 1999).This could be an indication for FGF8 to be an inducer. We propose the involvement of FGF8 in the induction of VEGFR-2 and in endothelial cell formation. Thus, it is possible that FGF-8 does not only act as an autocrine mitogenic factor but also as a paracrine angiogenic factor. Previous in vivo and in vitro work has suggested that the VEGF and FGF signaling systems, acting through distinct receptor kinases, may function in a synergistic manner to enhance angiogenesis and tumorigensis (Pepper et al., 1992; Goto et al., 1993; Asahara et al., 1995). It was shown that androgens also induces the expression of VEGF (Ruohola et al., 1999), moreover, FGF8 was originally identified as an androgen-induced growth factor (Tanaka et al., 1992). FGF8 is thus a potential candidate for synergistic action with VEGF. However, the possibility of the cooperation of VEGF and FGF8 in Quek1 regulation remains to be studied. Together, these data indicate that FGF8 signaling cooperates with BMP4 signaling from IM and/or LPM to induce Quek1 expression in somites, similar to their involvement in the regulation of avian cardiogenesis (Alsan and Schultheiss, 2002).

As somites mature along the cranio-caudal axis, expression of GATA2, SCL/TAL1, VEGFR2 and QH1 become localized in a more lateral and ventral position (Pouget et al., 2006). Immunohistological analyses showed that vascular smooth muscle (VSM) cells and pericyte precursors colonize the aorta from the medioventral aspect of the somite, in close association with the sclerotomal compartment. Similarly from HH-stage 15 onwards, expression of Quek1 was also observed in sclerotome cells surrounding the neural tube (Fig. 1C), where it becomes stronger during later stages (Fig. 1D) which may contribute to endothelial cells that can replace hemogenic endothelium lining the aortic floor and can express α-smooth muscle actin. This depicts the relationship of endothelial remodelling with smooth muscle formation during aorta development (Pouget et al., 2006). We suggested earlier that BMP4 expressed in the neural tube, which is no longer antagonized efficiently by the decreasing level of Noggin in the notochord and in the dermomyotome, initiates Quek1 expression in sclerotome domain (Nimmagadda et al., 2005). We searched for other signals that are involved in the formation of this domain in the medial sclerotome cells surrounding. Wnt-1, 3a and 4 are expressed in the dorsal neural tube and have been shown to be regulators of medial somite patterning (Roelink and Nusse, 1991; Parr et al., 1993; Hollyday et al., 1995; Munsterberg et al., 1995; Marcelle et al., 1997; Ikeya et al., 1997; Geetha-Loganathan et al., 2006). Previous studies have shown that Wnt/β-catenin signaling promotes angiogenesis in vitro (Masckauchan et al., 2005; Wang et al., 2006). We found that grafting of Wnt 1 or 3a-expressing cells results in an up-regulation of Quek1 expression. Both positive and negative regulators within the canonical Wnt signaling pathway were preferentially expressed in embryonic stem cell-derived Flk1+ cells and in human primary endothelial (HUVEC, HMVEC, HUAEC) cells, suggesting a significant role of Wnt signaling in vascular development. Further, Wnt 3a and 2 (which activate the same canonical pathway) were able to expand the Flk+ cell population whereas treatment with Sfrps almost completely depleted the Flk+ cell population (Masckauchan et al., 2005; Wang et al., 2006). Similarly, we show that Wnt 1 and 3a can induce the increase in number of endothelial cells comparable with the region of Quek1 induction in somite, whereas over-expression of Sfrp-2 results in down-regulation of Quek1 expression and reduced number of endothelial cells in the somite. Consistent with our results, Wnt activation has been shown to be both necessary and sufficient for endothelial cell differentiation and assembly into vascular-like structures in embryoid bodies, in part through the regulation of differentiation

and proliferation of endothelial progenitors (Reya et al., 2000, 2003; Wang et al., 2006).

Furthermore, ectopic expression of stabilized forms of β-catenin or Wnt1or 3a can promote endothelial proliferation and survival of both mouse and human cell lines (Wright et al., 1999; Biswas et al., 2003; Wu et al., 2003; Maretto et al., 2003), and induces extensive capillary-like network (similar to our observation) as was observed in HUVEC (Masckauchan et al., 2005). This increase in capillary network formation may reflect a morphogenetic function of Wnt/β-catenin signaling in endothelial cells. In conflict with these reports and our results, Wnt-1 signaling was reported to inhibit HUVEC proliferation (Cheng et al., 2003) in co-culture of HUVEC and C57MG mammary epithelial cells. These cells were transformed by Wnt-1 and thus may express many different gene products that may influence endothelial cell growth. A role of Wnt-1 signaling in tumorigenesis has been shown, to induce transformation of mammary cell line (Rijsewijk et al., 1987), of chicken embryonic and Rat-1 fibroblasts by β-catenin (Aoki et al., 1999; Young et al., 1998) and interference with TCF/β-catenin signaling leads to inhibition of proliferation in tumor cells (Stockinger et al., 2001; van de Wetering et al., 2003; Lepourcelet et al., 2004). Wnts are known to cooperate specifically with FGFs in their angiogenic activities like the promotion of mammary tumors in the mouse model (MacArthur et al., 1995). A similar type of cooperation may also occur in the process of Quek1 expression.

Our data show that one of the roles of BMP4/FGF8 from the IM and Wnt-1/3a from the neural tube is to induce Quek1 expression in the lateral and medial portion of the somite respectively. Interestingly, BMP4 and Wnt-1/3a are also expressed in the neural tube but the medial somite remains Quek1 negative. This can be explained by the BMP antagonizing role of the notochord which is mediated by noggin and Wnt antagonizing Sfrp-2 in the neural tube. Sfrp-2 is expressed throughout the developing neural tube during early stages of chick development (till HH-stage 9). By stage 12, it is restricted to the posterior neural tube and expressed throughout the dermomyotome. By HH-stage 20, expression of Sfrp-2 in the neural tube is completely lost, but strong in the dorsal medial lip of the dermomyotome in all somites (Ladher et al., 2000). Noggin expression in the notochord and in the dermomyotome (Nimmagadda et al., 2005) and Sfrp-2 expression in neural tube and in the dermomyotome (Ladher et al., 2000), is gradually decreasing at later stages, allowing the initiation of Quek1 expression in sclerotome cells, which surround the neural tube. This expression is becoming stronger during later stages of sclerotome maturation (Nimmagadda et al., 2004), which may correspond to a reduced level of noggin and Sfrp-2. Appearance of Quek1 expression in the notochord at day 4 correlates with the loss of noggin expression in the notochord. Then, BMP4 produced by the neural tube is no longer antagonized to block its effect in inducing Quek1 expression. In the compartmentalized somite, expression of Quek1 is present in the lateral portion of dermomyotome and sclerotome. This is due to the inducing effect of BMP4 and FGF8 produced and secreted by the IM (Fig. 4).

To conclude, we present a comprehensive model on the regulation of VEGFR-2 (Quek1) expression in the somite, in which the induction of Quek1 expression and endothelial cell formation in the somites are mediated by a cooperative action of BMP4, FGF8, Wnt1 and Wnt3a.
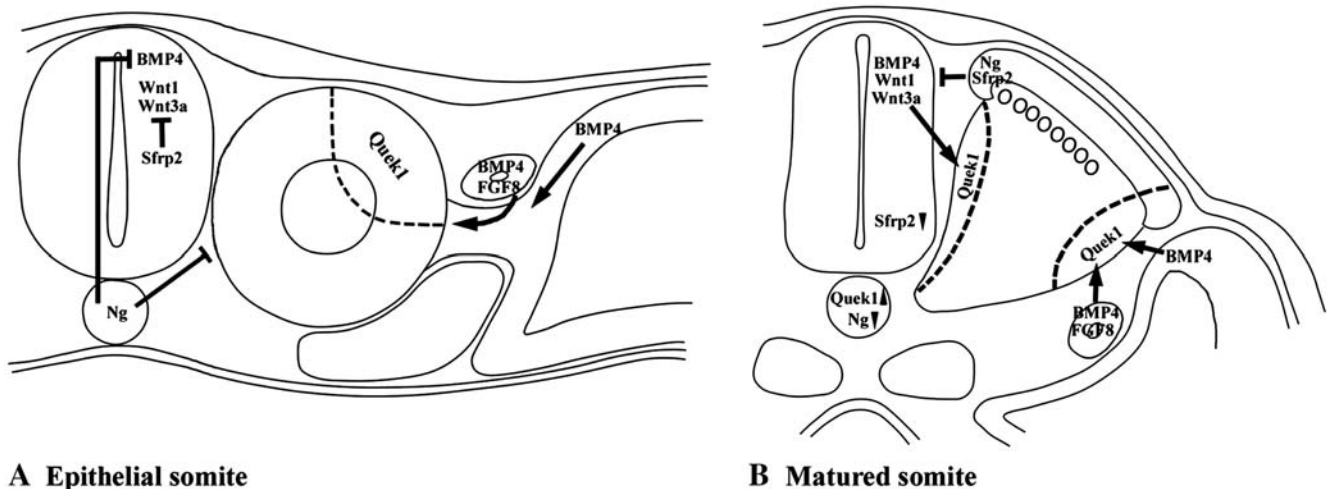

Fig. 4. An improved model for the regulation of Quek1 expression in the somite. Arrows indicate positive inductions, arrowheads indicate either up-regulation/down-regulation, while lines with bars represent inhibitory actions. (A) Epithelial somite: The expression of Quek1 is restricted to the lateral portion of the somite. BMP4 and FGF8 produced in the IM induce the expression of Quek1 in the lateral portion of the somite. BMP4 and Wnts are also present in dorsal neural tube but the expression of Quek1 is not seen in the medial half of somite. Noggin produced by the notochord and Sfrp-2 in the neural tube is thought to antagonize BMP4 and Wnts medially. (B) Compartmentalized somite: Quek1 is expressed in the lateral portion of dermomytome and sclerotome. From HH-stage 15 onwards, Quek1 becomes expressed in the sclerotome cells surrounding the neural tube. BMP4 and Wnts in the neural tube induce Quek1 expression in the medial somite due to the down-regulation of Sfrp and Noggin expression in the axial organs. Expression of Quek1 is also seen in the notochord from day 4 onwards. Later, noggin expression in the notochord is down-regulated allowing BMP4 from the dorsal neural tube to induce Quek1 expression in the sclerotome cells surrounding the neural tube. Quek1 is not expressed in the medial dermomyotome, because BMP4 and Wnts are dorsally antagonized by noggin and Sfrp produced in the dorsal dermomyotomal lip. In the notochord, the initiation of Quek1 expression corresponds to the down-regulation of noggin.

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