Differential expression of KDR/VEGFR-2 and CD34 during mesoderm development of the early human embryo

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Gene expression pattern

Differential expression of KDR/VEGFR-2 and CD34 during mesoderm development of the early human embryo

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Abstract

Recent findings on vertebrate embryos have provided compelling evidence for the existence of hemangioblasts, i.e. common precursors for endothelial and hematopoietic cells, characterized by expression of the VEGFR2/Flk1 receptor. We describe here a population of KDR^+ $CD34^-$ mesoderm cells that emerges in early-somitic human embryos, by the beginning of the 4th week of gestation. In the developing blood vessels, KDR-expressing $CD34^-$ cells gradually coexpress increasing levels of CD34 antigen. Remarkably, as development proceeds, a KDR^+ $CD34^-$ contingent persists in the paraaortic splanchnopleura until just prior to the emergence of aorta-associated hematopoietic cell clusters. These observations suggest that KDR^+ $CD34^-$ mesodermal cells might represent the putative hemangioblastic precursor of human hematopoietic and endothelial lineages.

Keywords: VEGFR-2; KDR; CD34; Mesoderm; Hematopoietic stem cell; Endothelium; Human embryo

1. Introduction

Converging experimental data designate the VEGF receptor tyrosine kinase Flk-1/VEGFR-2 as a pivotal regulator of endothelial and hematopoietic development from a common precursor, the hemangioblast (Sabin, 1920). In mice, targeted inactivation of the *flk-1* gene is lethal at mid-somitic stages and results in a complete block in both vascular and hematopoietic development (Shalaby et al., 1995, 1997). This dual defect fits with the recent characterization, in avian and mouse models, of $VEGFR\text{-}2^+$ mesodermal precursors able to generate both cell types in vitro (Eichmann et al., 1997; Choi et al., 1998; Nishikawa et al., 1998).

We have previously reported that KDR, the homologue of VEGFR-2/Flk1 (Terman et al., 1992), is highly expressed in the 5-week human embryo by endothelial cells but barely detectable in the first hematopoietic stem cells arising in the wall of the aorta (Labastie et al., 1998). Conversely, the CD34 protein is shared by both cell types from the stage of blood-island differentiation in the yolk sac (Civin et al., 1984; Fina et al., 1990; Tavian et al., 1996; Labastie et al., 1998). We thought to take advantage of this differential expression pattern to trace the emergence of putative human hemangioblasts and their segregation into endothelial and hematopoietic lineages.

Hybridization of sagittal and cross-sections of early 4-week embryos (stage 10, 4–12 somites) revealed several domains of KDR expression within the mesodermal layer. While low amounts of KDR messengers were detected in the cephalic region (Fig. 1B), endocardial tubes within the heart primordia and a stripe of lateral mesoderm cells in the trunk were intensely stained (Fig. 1B,E). In the posterior region, high amounts of KDR transcripts were detected in the ventral mesoderm near the base of the allantoic stalk (Fig. 1A,C,E). Immunostaining of alternate embryo sections revealed that none of the KDR^+ cells within the intraembryonic mesoderm layer co-expressed the CD34 protein at this early developmental stage (Fig. 1F–H).

KDR-expressing cells were also observed, either isolated or assembled in pre-endothelial cords in the presumptive course of the main blood vessels of the embryo. Caudal to the heart primordia, increasingly abundant stretches of

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KDR-expressing cords were present in the space between the embryonic endoderm and mesoderm (Fig. 1E and data not shown). From their localization, it can be inferred that these strands will later fuse and form the dorsal aortae and vitelline vessels. When analyzed for CD34 expression, cord-like structures of endothelial progenitors located between the intraembryonic mesoderm and endoderm layers were found to consist of KDR^+ $CD34^+$ cells (Fig. 1H and data not shown). In contrast, in the presumptive umbilical vein region, two sorts of KDR^+ cells were distinguishable: small clusters of 2–3 KDR-expressing angioblasts (Fig. 2A,E) which were weakly positive for CD34 expression (Fig. 2B,G), and flattened endothelial cells surrounding an incipient vascular lumen, that displayed strong KDR and CD34 staining (Fig. 2C,F and D,H). Thus, blood vessel differentiation seemed to correlate with CD34

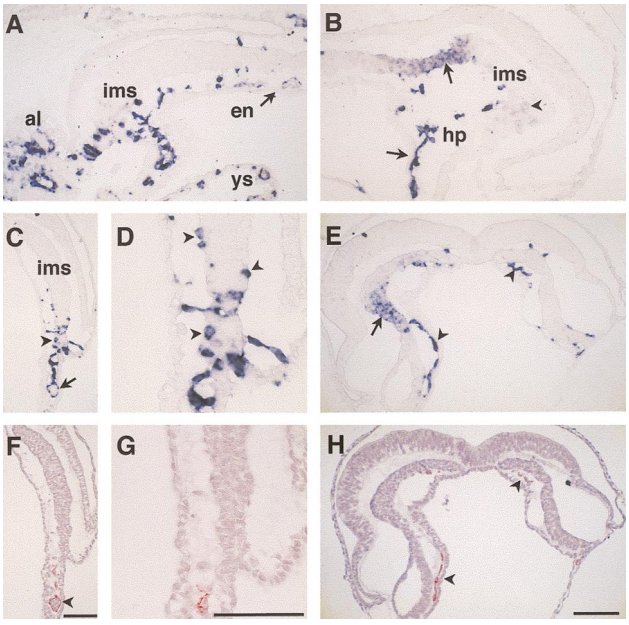

Fig. 1. Analysis of KDR and CD34 expression in early-somitic human embryos. (A–E) In situ hybridization of a digoxygenin-labeled KDR probe on sections of 6- and 7-somite human embryos. Dorsal is up in all images. In (A) and (B), anterior is on the right. (A). In the posterior region, KDR-expressing cells are present in the allantoic stalk and intraembryonic mesoderm, as well as between the endodermal and mesodermal layers. (B) Cephalic and trunk region. Weak expression on head mesoderm (arrowhead), contrasts with high levels detected in trunk mesoderm and endocardium cells within the heart primordium (arrows). (C–E). Transverse sections at the posterior and trunk levels. (C) Posteriorly, KDR is found on ventral mesoderm (arrowhead) and neighbouring blood vessels connecting the embryo with the yolk sac (arrow). (D) High magnification of (E); stained cells are present within the mesoderm layer and often seem to bud towards the adjacent spaces (arrowheads). (E) In the trunk region, KDR is expressed in intermediate/lateral mesoderm (arrow), as well as on cell cords close to the endoderm (arrowheads). (F–H) CD34 immunostaining of sections adjacent to those in (C–E), hematoxylin counterstaining. CD34 and KDR are coexpressed in forming blood vessels (arrowheads), but all KDR-expressing cells within the mesodermal layer are CD34-negative. Magnification: (A,B,E,H $\times$ 110; C,F $\times$ 80; D,G $\times$ 220. Scale bars, 100 μm. al, Allantoic stalk; en, endoderm; hp, heart primordium; ims, intraembryonic mesoderm; ys, yolk sac.

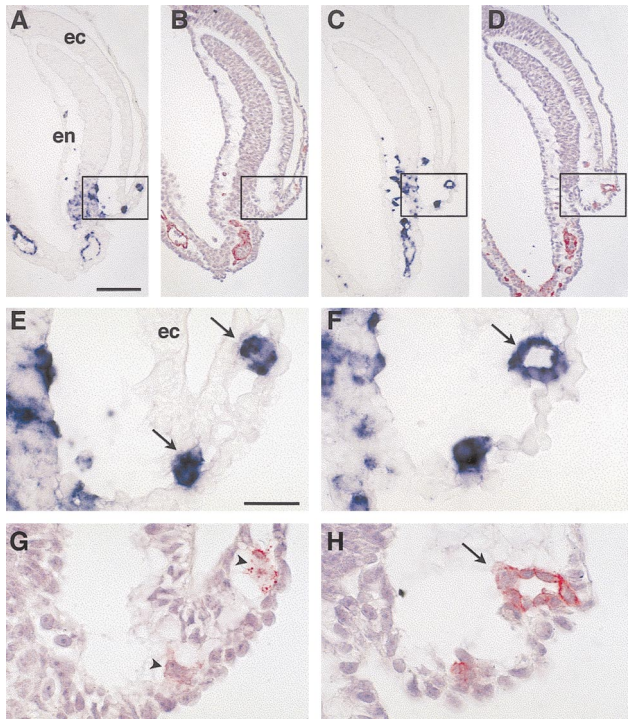

Fig. 2. Serial cross-sections of a 6-somite embryo stained for KDR and CD34 expression in the presumptive umbilical vein territory (framed areas). (A–D) Low magnification views of the right half of the embryo showing the umbilical vein site at caudal (A,B) and posterior trunk levels (C,D). (E) Close-up view of (A); arrows point to 2-cell clumps displaying strong KDR expression. (F) High power magnification of (C) showing a vascular structure with elongated KDR^+ cells surrounding a luminal space (arrow). (G,H) CD34 immunostaining of sections adjacent to those in (E,F) reveals weak labeling on aggregating angioblasts (G, arrowheads), but a strong one on cells exhibiting a typical endothelial morphology at a more anterior level (H, arrow). Magnification: A–D, $\times$ 110; E–H, $\times$ 550. Scale bars: A–D, 100 μm; E–H, 25 μm. ec, Ectoderm; en, endoderm.

expression by KDR^+ angioblasts. Consistent with this, the endothelial wall of umbilical veins and arteries, which were more developed in the allantoic stalk, already expressed high levels of KDR and CD34 at this stage (not shown), as did the whole intraembryonic vasculature at subsequent stages (Fig. 3).

Later in the 4th gestation week (stages 11 and 12, 13–29 somites), undifferentiated KDR^+ $CD34^-$ cells were found within the splanchnic mesoderm, i.e. the presumptive territory of intraembryonic blood cell formation, at the level of the hepatic diverticulum (Fig. 4A,B). By the end of the 4th week (28 somites), KDR^+ $CD34^-$ cells had dramatically increased in number within the splanchnic mesoderm (Fig. 4C,D). Interestingly, no such cells could be found beyond this stage, which shortly precedes the emergence of $CD34^+$ blood progenitors in the dorsal aorta. In contrast, all KDR^+ cells within the somatopleura co-expressed the CD34 protein (not shown), indicating that a primitive population of KDR^+ $CD34^-$ cells expands selectively within the splanchnic, but not somatic, mesoderm throughout the 4th week of development. This is consistent with previous studies in

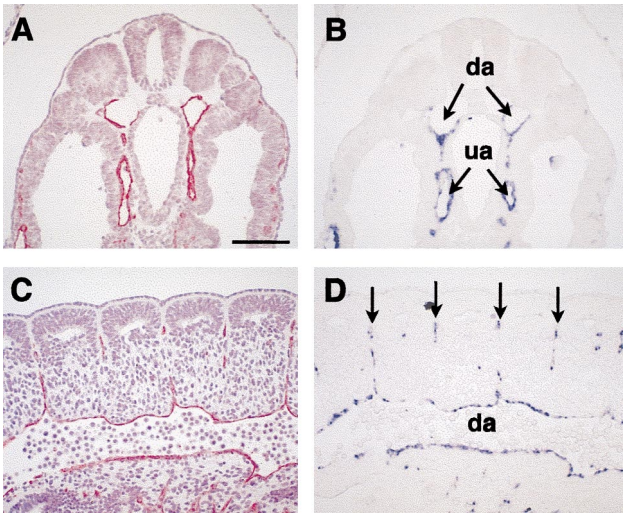

Fig. 3. Co-localization of KDR (B,D) and CD34 (A,C) in the whole vasculature of 13- to 28-somite embryos. (A,B) At the 13-somite stage (mid-4th week), all of the main blood vessels, such as the dorsal aortae and umbilical arteries coexpress KDR and CD34. (C,D) Twenty-five-somite embryo; endothelial cells in vessels formed by sprouting from pre-existing ones, such as intersomitic arteries (arrows) which arise from the dorsal aorta, are also KDR$^+$ CD34$^+$. Magnification: $\times$ 110. Scale bar, 100 μm. da, Dorsal aortae; ua, umbilical arteries.

avian and murine embryos showing that the splanchnic mesoderm is the territory of intraembryonic blood cell formation (Cumano et al., 1996; Pardanaud et al., 1996)

In summary, the expression patterns of KDR and CD34 in the human embryo are consistent with previous reports in the avian and murine models (Eichmann et al., 1993; Millauer et al., 1993; Yamaguchi et al., 1993; Young et al., 1995; Wood et al., 1997) and further suggest that KDR$^+$ CD34$^-$ cells might represent mesodermal precursors for the hematopoietic and endothelial lineages in man.

2. Experimental procedures

Human embryos were obtained with informed consent after voluntary terminations of pregnancy performed in compliance with the French legislation. Developmental stages were estimated on anatomic criteria, according to the Carnegie staging system (O'Rahilly and Müller, 1987).

Protocols for tissue processing, immunostaining, synthesis of KDR sense and antisense ribroprobes and in situ hybridization have been detailed elsewhere (Labastie et al., 1998). No staining was observed when irrelevant primary antibodies or a sense KDR riboprobe were used as controls.

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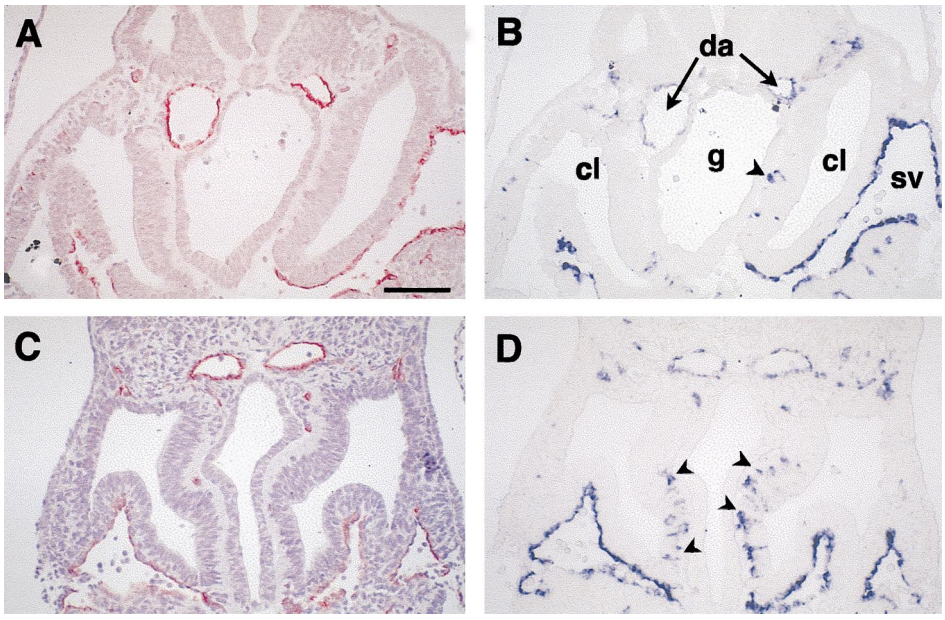

Fig. 4. KDR$^+$ CD34$^-$ cells within the paraaortic splanchnopleura of mid- (A,B) and late-4th week (C,D) human embryos. (A,B) In a 15-somite embryo, rare KDR-expressing cells in the splanchnic wall of the coelom (B, arrowhead) are CD34-negative (A), whereas the endothelial linings of paired dorsal aortae and sinus venosus express both markers. (C,D) In a 28-somite embryo, i.e. by the end of the 4th week of gestation, a much higher number of KDR$^+$ CD34$^-$ undifferentiated cells (arrowheads in D) is observed in the splanchnopleura neighbouring the hepatic primordium. Note that differentiated blood vessels express both markers. Magnification: $\times$ 110. Scale bar, 100 μm. cl, Coelom; da, dorsal aortae; g, gut; sv, sinus venosus.

References

Choi, K., Kennedy, M., Kazarov, A., Papadimitriou, J.C., Keller, G., 1998. A common precursor for hematopoietic and endothelial cells. Development 125, 725–732.

Civin, C.I., Strauss, L.C., Brovall, C., Fackler, M.J., Schwartz, J.F., Sharper, J.H., 1984. Antigenic analysis of hematopoiesis. III. A hematopoietic progenitor cell surface antigen defined by a monoclonal antibody raised against KG-1a cells. J. Immunol. 133, 157–165.

Cumano, A., Dieterlen-Lièvre, F., Godin, I., 1996. Lymphoid potential, probed before circulation in mouse, is restricted to caudal intraembryonic splanchnopleura. Cell 86, 907–916.

Eichmann, A., Marcelle, C., Bréant, C., Le Douarin, N.M., 1993. Two molecules related to the VEGF receptor are expressed in early endothelial cells during avian embryonic development. Mech. Dev. 42, 33–48.

Eichmann, A., Corbel, C., Nataf, V., Vaigot, P., Bréant, C., Le Douarin, N.M., 1997. Ligand-dependent development of the endothelial and hemopoietic lineages from embryonic mesodermal cells expressing vascular endothelial growth factor receptor 2. Proc. Natl. Acad. Sci. USA 94, 5141–5146.

Fina, L., Molgaard, H.V., Robertson, D., Bradley, N.J., Monaghan, P., Delia, D., Sutherland, D.R., Baker, M.A., Greaves, M.F., 1990. Expression of the CD34 gene in vascular endothelial cells. Blood 75, 2417–2426.

Labastie, M.C., Cortés, F., Roméo, P.H., Dulac, C., Péault, B., 1998. Molecular identity of hematopoietic precursor cells emerging in the human embryo. Blood 92, 3624–3635.

Millauer, B., Wizigmann-Voos, S., Schnurch, H., Martinez, R., Moller, N.P., Risau, W., Ullrich, A., 1993. High affinity VEGF binding and developmental expression suggest Flk-1 as a major regulator of vasculogenesis and angiogenesis. Cell 72, 835–846.

Nishikawa, S.I., Nishikawa, S., Hirashima, M., Matsuyoshi, N., Kodama, H., 1998. Progressive lineage analysis by cell sorting and culture identifies FLK1$^+$VE-cadherin$^+$ cells at a diverging point of endothelial and hemopoietic lineages. Development 125, 1747–1757.

O'Rahilly, R., Müller, F., 1987. Developmental stages in human embryos. Carnegie Institution of Washington, Washington. 306 pp.

Pardanaud, L., Luton, D., Prigent, M., Bourcheix, L.M., Catala, M., Dieterlen-Lièvre, F., 1996. Two distinct endothelial lineages in ontogeny, one of them related to hemopoiesis. Development 122, 1363–1371.

Sabin, F.R., 1920. Studies on the origin of blood vessels and of red blood corpuscles as seen in the living blastoderm of chicks during the second day of incubation. Carnegie Inst. Wash. Pub. no. 272. Contrib. Embryol. 9, 214–262.

Shalaby, F., Rossant, J., Yamaguchi, T.P., Gertsenstein, M., Wu, X.F., Breitman, M.L., Schuh, A.C., 1995. Failure of blood-island formation and vasculogenesis in Flk-1-deficient mice. Nature 376, 62–66.

Shalaby, F., Ho, J., Stanford, W.L., Fischer, K.D., Schuch, A.C., Schwartz, L., Bernstein, A., Rossant, J., 1997. A requirement for Flk-1 in primitive and definitive hematopoiesis and vasculogenesis. Cell 89, 981–990.

Tavian, M., Coulombel, L., Luton, D., San Clemente, H., Dieterlen-Lièvre, F., Péault, B., 1996. Aorta-associated CD34$^+$ hematopoietic cells in the early human embryo. Blood 87, 67–72.

Terman, B.I., Dougher-Vermazen, M., Carrion, M.E., Dimitrov, D., Armellino, D.C., Gospodarowicz, D., Bohlen, P., 1992. Identification of the KDR tyrosine kinase as a receptor for vascular endothelial cell growth factor. Biochem. Biophys. Res. Commun. 187, 1579–1586.

Wood, H.B., May, G., Healy, L., Enver, T., Morriss-Kay, G.M., 1997. CD34 expression patterns during early mouse development are related to modes of blood vessel formation and reveal additional sites of hematopoiesis. Blood 90, 2300–2311.

Yamaguchi, T.P., Dumont, D.J., Conlon, R.A., Breitman, M.L., Rossant, J., 1993. flk-1, an flt-related receptor tyrosine kinase is an early marker for endothelial cell precursors. Development 118, 489–498.

Young, P.E., Baumhueter, S., Lasky, L.A., 1995. The sialomucin CD34 is expressed on hematopoietic cells and blood vessels during murine development. Blood 85, 96–105.