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Differentiation of hemangioblasts from embryonic mesothelial cells? A model on the origin of the vertebrate cardiovascular system

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Abstract The existence of the hemangioblast, a common progenitor of the endothelial and hematopoietic cell lineages, was proposed at the beginning of the century. Although recent findings seem to confirm its existence, it is still unknown when and how the hemangioblasts differentiate. We propose a hypothesis about the origin of hemangioblasts from the embryonic splanchnic mesothelium. The model is based on observations collected from the literature and from our own studies. These observations include: (1) the extensive population of the splanchnic mesoderm by mesothelial-derived cells coinciding with the emergence of the endothelial and hematopoietic progenitors; (2) the transient localization of cytokeratin, the main mesothelial intermediate filament protein, in some embryonic vessels and endothelial progenitors; (3) the possible origin of cardiac vessels from epicardial-derived cells; (4) the origin of endocardial cells from the splanchnic mesoderm when this mesoderm is an epithelium; (5) the evidence that mesothelial cells migrate to the hemogenic areas of the dorsal aorta. (6) Biochemical and antigenic similarities between mesothelial and endothelial cells. We suggest that the endothelium-lined vascular system arose as a specialization of the phylogenetically older coelomic cavities. The origin of the hematopoietic cells might be related to the differentiation, reported in some invertebrates, of coelomocytes from the coelomic epithelium. Some types of coelomocytes react against microbial invasion and other types transport respiratory pigments. We propose that this phylogenetic origin is recapitulated in the vertebrate ontogeny and explains the differentiation of endothelial and blood cells from a common mesothelial-derived progenitor.

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Introduction

The early vascularization of the amniote embryo is a fast process. In only 48–72 h, the mouse embryo shifts from being completely avascular to a stage at which a well-developed network of blood-containing vessels supplies almost all the organism [51]. The endothelial cells and the blood cells are thought to originate from a hypothetical embryonic progenitor, the hemangioblast, whose existence was postulated at the beginning of this century [31, 49]. The hemangioblast hypothesis is supported by the sharing of molecular markers in cells with endothelial or hemopoietic potential [18, 35, 44, 50, 64, 75, 76]. Potential hemangioblastic cells have been isolated from embryonic stem cell-derived embryonic bodies [5].

After the hypothetical segregation of the angioblastic and hemogenic lineages, the endothelial progenitors (angioblasts) coalesce to form a capillary plexus in the splanchnic mesoderm (a process known as vasculogenesis). Other parts of the embryo, namely the neuroectodermal and somatopleural areas, are basically vascularized through angiogenesis, i.e., sprouting and growth of preexisting vessels [57].

The molecular mechanisms of blood vessel formation are being unveiled by some recent findings (reviewed in [1, 29, 61, 62]). However, a key question still exists: when and how is a mesodermal cell programmed to become an angioblast or, eventually, an hemangioblast? [13, 14, 16].

Some reports have suggested that commitment of cells to the hemangioblastic lineage occurs very early, during the formation of the mesoderm (primitive streak stage). This is based on experiments with quail blastodisc cells treated with basic fibroblast growth factor. These cells express the early hemangioblastic marker vascular endothelial growth factor receptor-2 (VEGFR-2) and form blood islands [22, 23]. These findings can be interpreted as differentiation of hemangioblasts from pluripotential epiblastic cells.

Our observations, especially of the development of the cardiac vessels, have suggested an alternative working hypothesis, which does not necessarily exclude the above-mentioned. We think that an epithelial-mesenchymal transition gives rise to a secondary mesenchyme from the splanchnic mesothelium at developmental stages E8–E11 in mice and E2–E4 in chick embryos. A subset of these mesothelial-derived cells would give rise to hemangioblasts and, subsequently, to the early embryonic vessels.

Some of the observations which substantiate this hypothesis have been published by us, but most of them have been collected from the literature. The aim of this paper is to gather this dispersed information and to propose a testable model for the differentiation of the hemangioblastic lineage from their mesodermal progenitors.

Methods

The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institute of Health (NIH publication No. 8523, revised 1985).

The sample studied consisted of embryos of dogfish (*Scyliorhinus canicula*), chick (*Gallus gallus*), quail (*Coturnix coturnix*), and Syrian hamster (*Mesocricetus auratus*). The Syrian hamster embryo is a mammalian model currently used in our laboratory. Although the gestation period is somewhat shorter than that of the mouse embryo, the developmental stages of both species are very similar by the time of the early development of the vascular system (8–11 dpc) [20].

Histomorphology

Fertilized dogfish eggs were obtained from adult females collected in the Bay of Malaga (western Mediterranean) by commercial trawling vessels. The eggs were kept in indoor tanks of well-aerated sea water. Whole embryos were fixed by immersion for 2–3 h in freshly prepared 1.25% glutaraldehyde and 1% paraformaldehyde diluted in elasmobranch buffer (16.38 g/l NaCl, 0.89 g/l KCl, 1.11 g/l $CaCl_2$, 0.38 g/l $NaHCO_3$, 0.06 g/l NaH_2PO_4, 21.6 g/l urea, pH 7.2). The fixed embryos were washed in elasmobranch buffer, and postfixed in 1% OsO_4 in the same buffer for 2 h at 4° C. Quail embryos were fixed in 1:1 paraformaldehyde-glutaraldehyde in PBS, washed and postfixed in 1% OsO_4 for 90 min. After washing, dogfish and quail embryos were dehydrated in an ethanolic series finishing in acetone and embedded in araldite 502. Semithin (0.5 to 1-μm) sections were obtained in a Reichert UMO-2 ultramicrotome and stained with toluidine blue.

Immunohistochemistry

The hamster and avian embryos were fixed in 40% methanol, 40% acetone, and 20% distilled water for 8–12 h. After fixation, the embryos were dehydrated in an ethanolic series finishing in butanol, and paraffin-embedded. Serial 5-μm and 10-μm sections were obtained with a Leitz microtome and collected on poly-L-lysine coated slides.

The sections were dewaxed in xylene, hydrated in an ethanolic series and washed in Tris-phosphate buffered saline (TPBS, pH 7.8).

For immunohistochemistry, endogenous peroxidase activity was quenched by incubation for 30 min with 3% hydrogen peroxide in TPBS. After washing with TPBS, non-specific binding sites were saturated for 30 min with 10% sheep serum, 1% bovine serum albumin, 0.1% Triton X-100 in TPBS (SBT). The slides were then incubated overnight at 4° C in the primary antibody diluted in SBT. Control slides were incubated in SBT only.

After incubation, the slides were washed in TPBS, incubated for 1 h at room temperature in biotin-conjugated anti-mouse or anti-rabbit goat IgG (Sigma) diluted 1:100 in SBT, washed again and incubated for 1 h in avidin-peroxidase complex (Sigma) diluted 1:150 in TPBS. Peroxidase activity was developed with Sigma Fast 3,3′-diaminobenzidine tablets according to the indications of the supplier.

The monoclonal QH1 and anti-chick vimentin (clone AMF-17b) antibodies (Developmental Studies Hybridoma Bank, University of Iowa) were used at 1:500 and 1:200 dilution of the supernatants, respectively.

Two cytokeratin antibodies were used. Monoclonal anti-pan cytokeratin (C2562, Sigma, U.K.) is a mix of six monoclonal antibodies which stains most types of keratin in epithelial cells of several vertebrates, but it shows no cross-reaction with non-epithelial normal human tissues. It was used at 1:200 dilution. Polyclonal anti-cytokeratin (Z622, Dakopatts, Denmark) is used for wide screening of keratins in several tissues. This antibody was diluted at 1:500. No significant differences were found between the results obtained with both antibodies, but the monoclonal one gave less background staining.

For the CK/QH1 double immunolabelling, the slides were incubated overnight at 4° C with the polyclonal anti-CK antibody diluted in SBT. After washing in TPBS, the slides were incubated for 1 h in biotin-conjugated goat anti-rabbit IgG (Sigma) diluted 1:100 in SBT, washed and incubated for 1 h in extravidin-FITC conjugate (Sigma) diluted 1:150. The slides were blocked again in SBT for 30′, incubated for 1 h with the monoclonal QH1 antibody diluted in SBT, washed and incubated for 1 h in TRITC-conjugated goat anti-mouse IgG (Sigma) diluted 1:50 in SBT. After washing, the sections were observed in a laser confocal microscope Leica TCS-NT, using filters specific for the FITC and TRITC fluorochromes. Selected images were captured and printed in a Sony digital color printer.

Observational support of the hypothesis

1. The early splanchnic mesoderm seems to be populated by cells of mesothelial origin.

The splanchnic mesoderm of the E8.5–E11 mouse embryo and the E2.5–E4 chick embryo contains endothelial progenitor cells [7, 54, 57]. Furthermore, the earliest intraembryonic hematopoietic stem cells have been localized in the mesodermal area comprised between the dorsal mesentery, the dorsal aorta and the mesonephros [10, 13, 26, 28, 42, 43, 56]. We have obtained morphological and immunohistochemical evidence that mesothelial cells detach from their neighbours and migrate into the splanchnic mesoderm of those areas at precisely those developmental stages.

The mesothelium close to the endodermal tissues, dorsal aorta and mesonephros shows, by those developmental stages, morphological traits which suggest an epithelial-mesenchymal transition. The presence of increased mitotic figures, long cytoplasmic basal projections and reduced adhesion between the mesothelial cells is especially noticeable (Fig. 1A–C). However, at later stages or in other areas of the embryo, the mesothelium is formed by squamous, cubic or prismatic cells, without signs of migration.

We have observed these features in the dogfish embryo, a primitive vertebrate model, which suggests a de-

velopmental event highly conserved throughout the vertebrate evolution (Fig. 1A). During cardiac morphogenesis, an epithelial-mesenchymal transition of the epicardial mesothelium seems to occur in particular areas of the heart [41, 48, 58, 60]. The epicardial-derived cells might contribute to the coronary vasculature, as stated below.

Further evidence that mesothelium contributes to the splanchnic mesenchyme is based on immunohistochemical studies of the distribution of the intermediate filament proteins cytokeratin and vimentin.

Cytokeratin (CK) is a main component of the cytoskeleton of diverse embryonic and adult epithelia, including the mesothelium. On the other hand, it is known that CK transiently persists after the transdifferentiation of an epithelium into mesenchyme, as has been reported from both in vivo and in vitro systems [21, 30]. Thus, if mesothelial-derived cells populate the underlying mesodermal areas, they should retain CK immunoreactivity for some time.

On the other hand, the expression of vimentin (VIM) in an epithelial cell may be an indication of its transformation into a migratory, mesenchymal cell, as it has been demonstrated in the primitive streak and neural tube [25]. Vimentin expression is probably involved in the premigratory shape changes [30, 53]. Thus, if our hypothesis is correct, we should expect a transient colocalization of CK and VIM in the mesothelial cells as well as in the mesenchymal cells derived from them.

In fact, some studies [70, 73, 74], and our own observations [58, 60] have demonstrated this colocalization in the splanchnic mesothelium and mesenchyme by the time at which the vasculogenic and hematopoietic progenitors are present (Fig. 1D–F). The areas of colocalization include the epicardium, the liver, the dorsal mesentery and, particularly, the mesothelial areas closest to the dorsal aorta, where the morphological evidence of epithelial-mesenchymal transition is stronger (Fig. 1B, E). However, the mesenchyme from other areas of these embryos is, in general, cytokeratin-negative (Fig. 1D). From our observations, this CK immunoreactivity of the splanchnic mesenchyme is transient, and it disappears in later embryos. On the other hand, a weak VIM immunoreactivity persists in the adult mesothelium [11, 34] and increases significantly when mesothelial cells are cultured and acquire a mesenchymal phenotype [37]. In fact, adult mesothelial cells are unique in their ability to alter their phenotype and intermediate filament composition reversibly [8].

2. CK immunoreactivity can be transiently detected in early endothelial cells, and it colocalizes with vascular markers in putative angioblastic cells.

If we assume that CK immunoreactivity may persist for some time in mesothelial-derived cells, and if these cells have vasculogenic potential, it might be possible to detect CK remnants in immature endothelial cells of the forming blood-vessels or, at least, to colocalize CK with some early vascular markers. In fact, we have observed this transient CK immunoreactivity in some early endothelial cells of the dorsal mesentery, liver and cardiac vascular plexus of avian and hamster embryos (Fig. 1F). The CK immunoreactivity of the primitive endothelial cells is usually weak and persists for only a few fours, according to our estimations.

We have also reported colocalization of CK and the early specific hemangioblastic markers QH1 and VEGFR-2 in isolated subepicardial cells, which could be considered as the progenitors of the cardiac vessels [59, 60].

It is possible that the report of CK immunoreactivity in adult endothelial cells of lower vertebrates such as the rainbow trout [39] and the toad *Xenopus laevis* [32] can be regarded in this context, as we will discuss below.

3. Early cardiac vessels probably originate from epicardial mesothelial cells

We have published a study of the development of the primitive epicardium and the early stages of heart vascularization in a primitive vertebrate, the dogfish (*Scyliorhinus canicula*) [47, 48]. In these papers we concluded that: (1) The epicardium develops from mesothelial cells which are released in the pericardial cavity and adhere to the myocardial wall. (2) The epicardium first covers the atrioventricular and conoventricular grooves, and then it grows towards the arterial and venous poles of the heart. (3) The early subepicardial mesenchymal cells (SEMC) originate from the mesothelial epicardial cells. This transformation occurs in specific areas of the heart, mainly in the atrioventricular and conoventricular grooves. (4) Some of the early SEMC coalesce through junction complexes and form the primitive vascular structures of the subepicardium (blood islands, capillaries and veins). (5) Only when the epicardium covers the sinus venosus and makes contact with the splanchnic mesothelium, might mesenchymal cells be able to migrate from the liver area to the subepicardium. However, by this stage, there are already blood-containing capillaries and veins in the heart. Thus, this unique embryonic model of epicardial development allowed us to discard the possibility that the early cardiac vessels originate from angioblasts migrating from extracardiac areas, and we postulated a mesothelial origin for the precursors of the early cardiac vessels in this primitive vertebrate.

The origin of the subepicardial mesenchyme from the epicardial mesothelium through an epithelial-mesenchymal transition has been supported by recent findings using in vivo and in vitro models [12]. On the other hand, the hypothesis of an embryonic origin of the endothelium of the cardiac vessels from mesothelial-derived cells has been suggested by other authors [41]. This possibility has also been stressed by experimental studies [2]. Explants of quail embryo hearts onto collagen produced, during the first day in culture, an outgrowth of epithelial

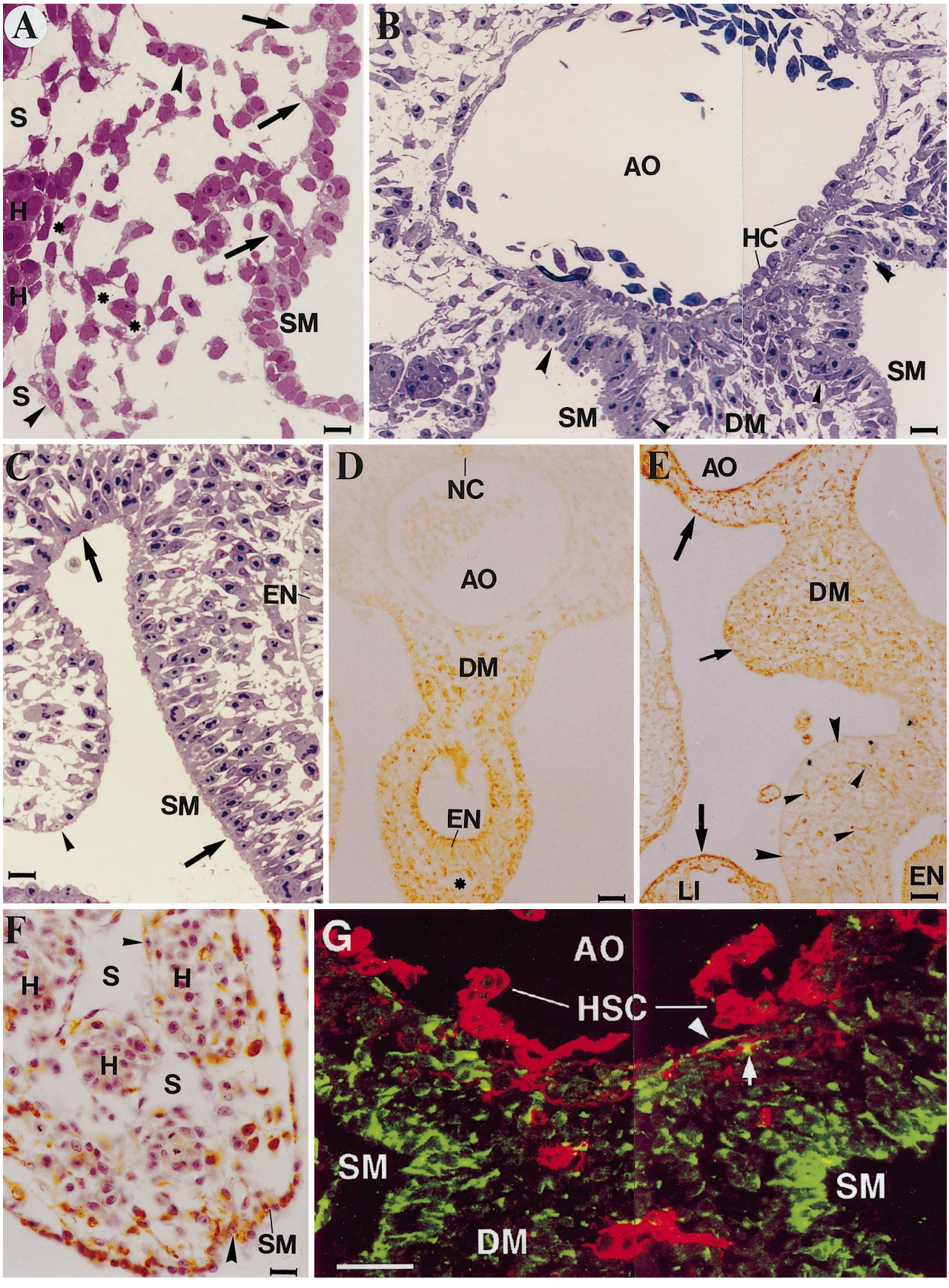
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Fig. 1A–G Morphological and immunohistochemical evidence of migration of mesothelial cells to the areas where endothelial and hematopoietic progenitors differentiate. **A** Dogfish embryo, 17 mm in total length, sagital section. This semithin section shows the mesenchyme which develops between the early hepatic tissue (*H*) and the heart (which is located in the *upper part*). Some splanchnic mesothelial cells (*SM*) show signs of ingression in the underlying space (*arrows*). Some cells (*arrowheads*), similar to the mesenchymal cells, are integrated in the endothelial lining of the hepatic sinusoids (*S*). Other mesenchymal cells are apparently forming ring-like structures (*asterisks*), which could be interpreted as developing vessels. *Scale bar* 12 μm. **B** Quail embryo, Hamburger and Hamilton (*HH*) stage 17, transverse section. The dorsal aorta (*AO*) shows the early stages of the differentiation of the intraaortic clusters of hematopoietic cells (*HC*), which are being released into the aortic lumen. The splanchnic mesothelium (*SM*) shows morphological evidence of migration of cells to the dorsal mesenterium (*DM*) and to the floor of the aorta. The migration of mesothelial cells seems to be more intense in the areas closest to the aorta (*large arrowheads*) than in the mesenterium (*small arrowheads*). *Scale bar* 15 μm. **C** Quail embryo, HH-stage 16, transverse section. In this stage, there is strong evidence of an epithelial-mesenchymal transition involving the splanchnic mesothelium (*SM*) in the areas close to the endoderm (*EN*). Note the long basal cytoplasmic processes (*arrows*) and the abundance of mitotic figures in the mesothelial lining. Compare with the mesothelium covering other areas of the embryo (*arrowhead*). *Scale bar* 12 μm. **D** Hamster embryo, 9.5 days post coitum, transverse section, cytokeratin (*CK*) immunostaining. The splanchnic mesenchyme from the dorsal mesentery (*DM*) and the areas surrounding the endoderm (*EN*) is CK-immunoreactive. This area of CK-positive cells shows a sharp limit coinciding with the floor of the aorta (*AO*). The notochord (*NC*) is also immunoreactive. Note the presence of developing vessels (*asterisk*) in the area populated by CK-immunoreactive cells. *Scale bar* 32 μm. **E** Chick embryo, HH-stage 20, oblique section between the transverse and the frontal planes. Vimentin (*VIM*) immunostaining. The splanchnic mesothelium shows different degrees of VIM immunoreactivity. The strongest immunoreactivity (*large arrows*) appears close to the dorsal aorta (*AO*) and in the liver (*LI*). The immunoreactivity in the dorsal mesenterium (*DM*) is heterogeneous (*small arrow*), and it appears greatly decreased (*large arrowheads*) in the mesothelium close to the endoderm (*EN*), where numerous vessels have already developed (*small arrowheads*). Thus, VIM expression seems to be down-regulated in the mesothelial cells from areas where the initial vasculogenetic process has finished. *Scale bar* 32 μm. **F** Quail embryo, HH-stage 17, transverse section. Cytokeratin immunostaining. The early stages of liver development are characterized by abundant CK-immunoreactive mesenchymal cells, which seem to originate from the splanchnic mesothelium (*SM*; *arrowhead*) and intermingle with the hepatic tissue (*H*). The involvement of these mesothelial-derived cells in the formation of the hepatic sinusoids (*S*) is suggested by the weak CK immunoreactivity of some endothelial cells (*arrowhead*). Endothelial cells were CK-negative in other areas of this embryo. *Scale bar* 12 μm. **G** Quail embryo, HH-stage 17, transverse section similar to that in **B**. Double cytokeratin (*green*)/QH1 (*red*) immunostaining. Laser confocal microscopy. CK-immunoreactive cells seem to be migrating from the splanchnic meosthelium (*SM*) to the ventral wall of the aorta (*AO*), where QH1-immunoreactive hematopoietic stem cells (*HSC*) are being released into the lumen. Note the abundance of CK-immunoreactive cells in the dorsal mesentery (*DM*) and, especially, in the area comprised between the mesothelium and the aortic wall. However, CK-immunoreactive mesenchymal cells are not present in more dorsal areas. Traces of CK/QH1 colocalization can be seen in the aortic wall (*arrow*), even in adluminal positions (*arrowhead*). *Scale bar* 20 μm

cells which extended from the cut ends of the explants onto the surface of the collagen lattice. The epithelial cells (identified as ''epicardial'' in [41]) generated a population of mesenchymal cells that migrated into the collagen lattice and interconnected in primitive vascular networks, which reacted positively with the monoclonal antibody QH-1, a marker of endothelial and hemopoietic precursors. Significantly, only limited mesenchyme formation was observed when hearts were explanted prior to the epicardial covering. We have obtained similar results when we grafted early quail proepicardia (the epicardial primordium) into the pericardial cavity of chick embryos. The grafts produced large patches of QH1-immunoreactive epicardium, subepicardial mesenchyme and vessels [60].

4. Endocardial endothelial cells differentiate from the splanchnic mesoderm through an epithelial-mesenchymal transition

The endocardial endothelial cells differentiate from the precardiac splanchnic mesoderm, and this process has been characterized as an epithelial-mesenchymal transition [40, 41, 65]. In fact, the splanchnic mesoderm, by the time the endocardium differentiates, is an epithelium, which will continue in later embryos as the splanchnic mesothelium. We think that the origin of the endocardium can be regarded as the earliest manifestation of the model which we are herein proposing, i.e. the differentiation of a vasculogenic population from a mesodermal epithelium, through localized events of epithelial-mesenchymal transition.

5. Mesothelial cells migrate to the hemogenic areas of the aortic wall

Clusters of cells, considered as the progenitors of the hemopoietic stem cells, have been reported in the lateroventral areas of the dorsal aorta of the chick embryo by 3–4 days of incubation [9, 15] as well as in the human embryo at 5 weeks of gestation [66]. Morphological [52] and immunohistochemical studies (in preparation) reveal a substantial migration of mesothelial-derived cells towards these hemogenic areas (Fig. 1B, H). In fact, in the avian embryo, CK-positive cells can be seen all the way from the closest splanchnic mesothelium (which is strongly vimentin-immunoreactive, as shown in Fig. 1E) to the lateroventral aortic wall. In the quail embryo, the expression of the hemangioblastic marker QH1 progressively increases in the neighbourhood of the aortic lumen, and traces of CK/QH1 colocalization occur (Fig. 1G). We think that the migration of precursors from the adjacent mesothelium explains the characteristic lateroventral localization of the hemopoietic progenitors in the aortic lumen. The origin of the intraembryonic hematopoietic progenitors from mesothelial-derived cells had already been proposed on a morphologi-

cal basis [52], but this proposal has been surprisingly overlooked in most reviews about the origin of the hemopoietic system.

In relation to extraembryonic hematopoiesis, it is important to note that the differentiation of the precursors of the blood islands in the mammalian yolk sac has been described as a localized proliferation of cells from the splanchnic mesoderm, when this mesoderm constitutes an epithelial-like sheet covering the endoderm [29]. Thus, a similar mechanism, the transformation of splanchnic epithelial cells into mesenchyme, could explain the differentiation of the extra- and intraembryonic hematopoietic progenitors.

6. Mesothelial cells show antigenic and biochemical similarities to endothelial cells

Adult mesothelial cells express a number of vascular markers, such as Von Willebrand factor and receptor-mediated uptake of acetylated low-density lipoprotein (Ac-LDL) [19, 63, 69], the vascular-cell adhesion molecule (VCAM-1) and the CD44 antigen [27, 46, 67]. From the biochemical point of view, mesothelial cells are similar to endothelial cells in arachidonate metabolism and production of prostaglandin I_2 [63], angiotensin-converting enzyme [6], and tissue-type plasminogen activator and plasminogen activator inhibitors [69]. It is not surprising that cultured adult mesothelial cells have been mistaken for microvascular endothelial cells, from which they can be safely differentiated by their angiogenic response ([6], reviewed in [69]). However, this difference has not been tested yet in embryonic mesothelial cells, which, according to the report quoted above [2], might show an angiogenic response in vitro.

Ontogeny and phylogeny of the vertebrate cardiovascular system

From the ontogenetical point of view, the coelomic mesothelium is a direct derivative of the splanchnic mesodermal epithelium, which forms after the splitting of the lateral plate. As we have quoted above, hemangioblasts probably arise from the splanchnic epithelial sheet, which covers the yolk sac [29], and the earliest endothelial cells also differentiate from the splanchnic mesodermal epithelium close to the foregut [40, 41, 65]. The question is: Does the coelomic mesothelium derived from the mesodermal epithelium retain its ability to transform into a pluripotential mesenchyme? In this context, the coelomic mesothelium of the early embryo might be regarded as a pluripotential mesodermal tissue, which would supply hemangioblasts, smooth muscle cells and fibroblasts to all the splanchnic areas in response to local signals. The notion of the mesothelium as mesoderm has been extended even to the adult peritoneum in order to explain the cellular variability of the primary tumors of the serous surfaces [17].

We think that the suggested relationship between the embryonic mesothelial cells and the components of the vascular system (smooth muscle cells, endothelium and blood cells) might reveal a deeper phylogenetic significance. In the coelomate invertebrates, the relationship between the coelom and the circulatory system is very close. The coelomic cavities furnish the animals with a primitive system of circulation of the extracellular fluids, allowing for the distribution of nutrients and the withdrawal of metabolic waste. In fact, a few eucoelomate phylla (sipunculans, ectoprocts, echinoderms) depend largely on the body cavity as a coelomic circulatory chamber [3]. The case of the nemerteans is remarkable. These animals have a system of vessels lined with an endothelial-like epithelium. Studies on the development of these animals have shown that the vessels form by hollowing of a solid mesodermal band (schizocoely), as do the coelomic cavities of spiralian coelomate organisms such as annelids. This fact together with histological and cytological similarities suggested that nemertine vessels ae coelomic homologues [68].

It is conceivable that the system of endothelial-lined vessels has originated through the progressive invasion of non-coelomic body cavities by migratory mesothelial-derived cells. The ancestry of the endothelium from the phylogenetically older mesothelium would explain the similarities between these cell types, as well as the presence of cytokeratin in the endothelial cells of lower vertebrates. Assuming this, we can now interpret the embryonic differentiation of mesothelial-derived cells into endothelial cells as the ontogenetic recapitulation of this phylogenetic origin.

Regarding the origin of the hematopoietic stem cells from mesothelial progenitors, it is important to emphasize that the coelomocytes of the invertebrates probably originate from dissociation of cells from the coelomic mesothelium [33, 38, 45, 71]. These coelomocytes differentiate in specialized lineages, which undertake functions similar to those of the vertebrate blood cells, i.e. defense against microbial aggression and transport of respiratory pigments. Coelomocyte response, alike blood cells, seems to be regulated by cytokines analogous to vertebrate interleukins [4].

Conclusions

From a functional point of view, mesothelial cells share many similarities with endothelial cells. They are mesodermal epithelia, constituted of flat cells joined by junction complexes and lining fluid-filled cavities. They share expression of cell adhesion molecules, secrete substances inside the cavity and regulate the transport between the cavity and the subepithelial layer. We think that these similarities are not casual, and that the endothelium-lined system of vessels appeared as a specialization of the phylogenetically more primitive coelomic investment. According to our hypothesis, a recapitulation of this origin can be tracked in the ontogeny of the vertebrate embryo.

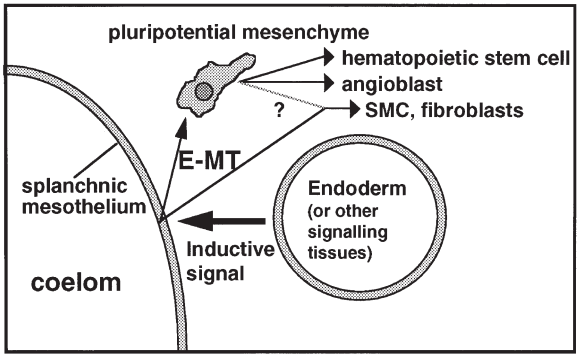

Fig. 2 Diagram of the proposed model of the differentiation of hemangioblasts in the vertebrate embryo. We suggest that the splanchnic mesothelium undergoes an epithelial-mesenchymal transition (*EM-T*) under the control of an inductive signal probably originating from the endoderm or, eventually, from other signalling tissues (dorsal aorta, myocardium). The mesothelial-derived mesenchyme would differentiate into hematopoietic, endothelial, smooth muscle (*SMC*) or fibroblastic cells. The definitive fate of the mesothelial-derived cells might be dependent on a preexisting commitment, regulated by the cell environment or by molecular interactions between successive populations of cells

The mesothelial model which we are proposing here is depicted in Fig. 2. We think that our observations as well as the quoted reports from the literature suggest an embryonic origin of hemangioblasts from the splanchnic mesothelium. The origin of smooth muscle cells and fibroblasts from mesothelial cells has also been suggested by recent findings [12].

A number of observations can be explained assuming the origin of vasculogenic cells from the splanchnic mesothelium. For example, the transient CK/VIM colocalization in the splanchnic mesoderm, the functional and antigenic similarities between endothelial and mesothelial cells, the sudden and massive differentiation of angioblasts throughout the splanchnic mesoderm and, finally, the close topological relationship between the vasculogenic and hematopoietic areas with the endoderm (which probably is the main inducer of the mesothelial transformation). Our model also may explain why some molecules which play a key role in epithelial-mesenchymal transitions are also markers of hemangioblastic progenitors. For example, the proto-oncogene *c-ets1*, which is involved in epithelial-mesenchymal transitions [16, 36, 72], is expressed by the splanchnic mesoderm and the immature endothelial and hemopoietic cells [55].

Our hypothesis is experimentally testable in several ways, which include the confirmation of the mesothelial origin of the CK-immunoreactive mesenchymal cells of the splanchnic mesoderm, the cell-lineage study of the mesothelial-derived population, culture of embryonic mesothelial cells in vitro, etc. On our laboratory, we are currently working in these directions.

If the mesothelial hypothesis is confirmed, many important questions will be raised. For example:

– What mechanism determines the fate of the mesothelial-derived cells as endothelial cell, blood cell, smooth muscle cell or other? Are the embryonic mesothelial cells committed prior to their shift to mesenchyme?
– What is the nature of the signalling system which induces the mesothelial transformation? Might this system be similar to that which drives other embryonic processes of epithelial-mesenchymal transition, including the mesoderm formation?
– Can the embryonic mesothelial-derived cells express their hemangioblastic potential in vitro? At what developmental stage is this potential lost?

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References

1. Beck L, D'Amore PA (1997) Vascular development: cellular and molecular regulation. FASEB J 11:3654–373
2. Bolender D, Olson LM, Markwald RR (1990) Coronary vessel vasculogenesis. In: Bockman DE, Kirby ML (eds) Embryonic origins of defective heart development. Ann NY Acad Sci 588:340–344
3. Brusca RC, Brusca GJ (1990) Invertebrates. Sinauer, Sunderland
4. Burke RD, Watkins RF (1991) Stimulation of star coelomocytes by interleukin-1. Biochem Biophys Res Commun 180:579–584
5. Choi K, Kennedy M, Kazarov A, Papadimitriou JC, Keller G (1997) A common precursor for hematopoietic and endothelial cells. Development 125:725–732
6. Chung-Welch N, Patton WF, Ameia Yen-Patton GP, Hechtman HB, Shepro D (1989) Phenotype comparison between mesothelial and microvascular endothelial cell lineages using conventional endothelial cell markers, cytoskeletal protein markers and in vitro assays of angiogenic potential. Differentiation 42:44–53
7. Coffin JD, Harrison J, Schwartz S, Hermark R (1991) Angioblast differentiation and morphogenesis of the vascular endothelium in the mouse embryo. Dev Biol 148:51–62
8. Connell ND, Rheinwald JG (1983) Regulation of the cytoskeleton in mesothelial cells: Reversible loss of keratin and increase in vimentin during rapid growth in culture. Cell 34: 245–253
9. Cormier F, Dieterlen-Lièvre F (1988) The wall of the chick embryo aorta harbours M-CFC, G-CFC, GM-CFC and BFU-E. Development 102:279–285
10. 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
11. Czernobilsky R, Moll R, Levy R, Franke WW (1985) Co-expression of cytokeratin and vimentin filaments in mesothelial, granulosa and rete ovarii cells of the human ovary. Eur J Cell Biol 37:175–190
12. Dettman RW, Denetclaw W, Ordahl CP, Bristow J (1998) Common epicardial origin of coronary vascular smooth muscle, perivascular fibroblasts, and intramyocardial fibroblasts in the avian heart. Dev Biol 193:169–181

13. Dieterlen-Lièvre F (1995) Early ontogeny of the human hematopoietic system. C R Soc Biol 189:601–609
14. Dieterlen-Lièvre F, Godin I, Pardanaud L (1997) Where do hematopoietic stem cells come from? Int Arch Allergy Immunol 112:3–8
15. Dieterlen-Lièvre F, Martin C (1981) Diffuse intraembryonic hemopoiesis in normal and chimeric avian development. Dev Biol 88:180–191
16. Dieterlen-Lièvre F, Pardanaud L (1992) Embryonic development of blood vessels. In: Maragoudakis ME, Gullino P, Lelkes PI (eds) Angiogenesis in Health and Disease. Plenum Press, New York, pp. 1–16
17. Donna A, Betta P (1981) Mesodermomas: a new embryological approach to primary tumours of coelomic surfaces. Histopathology 5:31–44
18. Eichmann A, Corbel C, Nataf V, Vaigot P, Bréant C, Le Douarin NM (1997) Ligand-dependent development of the endothelial and hemopoietic cell lineages from embryonic mesodermal cells expressing vascular endothelial growth factor receptor 2. Proc Natl Acad Sci USA 94:5141–5146
19. Eid H, Larson DM, Springhorn JP, Attawia MA, Nayak RC, Smith TW, Kelly RA (1992) Role of epicardial mesothelial cells in the modification of phenotype and function of adult rat ventricular myocytes in primary coculture. Circ Res 71:40–50
20. England MA (1994) The human. In: Bard J (ed) Embryos. Color Atlas of Development. Whole Publishing, London, pp. 207–220
21. Fitchett JE, Hay ED (1989) Medial edge epithelium transforms to mesenchyme after embryonic palatal shelves fuse. Dev Biol 131:455–474
22. Flamme I, Breier R, Risau W (1995) Vascular endothelial growth factor (VEGF) and VEGF receptor 2 (flk-1) are expressed during vasculogenesis and vascular differentiation in the quail embryo. Dev Biol 169:699–712
23. Flamme I, Risau W (1992) Induction of vasculogenesis and hematopoiesis in vitro. Development 116:435–439
24. Folkman J, D'Amore PA (1996) Blood vessel formation: what is its molecular basis? Cell 87:1153–1155
25. Franke WW, Grund C, Kuhn C, Jackson BW, Illmensee K (1982) Formation of cytoskeletal elements during mouse embryogenesis. III. Primary mesenchymal cells and the first appearance of vimentin filaments. Differentiation 23:43–59
26. Garcia-Porrero J, Godin I, Dieterlen-Lièvre F (1995) Potential intraembryonic hemogenic sites at pre-liver stages in the mouse. Anat Embryol 192:425–435
27. Gardner MJ, Jones LM, Catterall JB, Turner GA (1995) Expression of cell adhesion molecules on ovarian tumour cell lines and mesothelial cells, in relation to ovarian cancer metastasis. Cancer Lett 91:229–234
28. Godin I, Dieterlen-Lièvre F, Cumano A (1995) Emergence of multipotent hemopoietic cells in the yolk sac and paraaortic splanchnopleura in mouse embryos, beginning at 8.5 days postcoitus. Proc Natl Acad Sci USA 92:773–777
29. Haar JL, Ackerman GA (1971) A phase and electron microscopic study of vasculogenesis and erythropoiesis in the yolk sac of the mouse. Anat Rec 170:199–224
30. Hay ED (1990) Epithelial-mesenchymal transitions. Sem Dev Biol 1:347–356
31. His W (1900) Lecithoblast and Angioblast der Wirbeltiere. Abhandl Math-Phys Ges Wiss 26:171–328
32. Jahn L, Fouquet B, Rohe K, Franke WW (1987) Cytokeratins in certain endothelial and smooth muscle cells of two taxonomically distant vertebrate species, *Xenopus laevis* and man. Differentiation 36:234–254
33. Jamieson BGM (1992) *Oligochaeta*. In: Harrison FW (ed) Microscopic Anatomy of Invertebrates, Vol. 7, Wiley-Liss, New York, pp. 217–322
34. La Rossa PJ, Rheinwald JG (1984) Coexpression of simple epithelial keratins and vimentin by human mesothelium and mesothelioma in vivo and in culture. Cancer Res 44:2991–2999
35. Lin G, Finger E, Gutiérrez-Ramos JC (1995) Expression of CD34 in endothelial cells, hematopoietic progenitors and nervous cells in fetal and adult mouse tissues. Eur J Immunol 1995 25:1508–1516
36. Macías D, Pérez-Pomares JM, García-Garrido L, Carmona R, Muñoz-Chápuli R (1988) Immunoreactivity of the ets-1 transcription factor correlates with areas of epithelial-mesenchymal transition in the developing avian heart. Anat Embryol 198:307–315
37. Mackay SA, Tracy RP, Craighead JE (1990) Cytokeratin expression in rat mesothelial cells in vitro is controlled by the extracellular matrix. J Cell Sci 95:97–107
38. Mano R (1964) The coelomic corpuscles and their origin in the freshwater bryozoan, *Lophopodella carteri*. Sci Rep Tokyo Kyoiku Dai 11(B):211–235
39. Markl J, Franke WW (1988) Localization of cytokeratins in tissues of the rainbow trout: Fundamental differences in expression pattern between fish and higher vertebrates. Differentiation 39:97–122
40. Markwald RR (1995) Overview: Formation and early morphogenesis of the primary heart tube. In: Clark EB, Markwald RR, Takao A (eds) Developmental mechanisms of heart disease. Futura, Armonk, pp. 149–155
41. Markwald RR, Eisenberg C, Eisenberg L, Trusk T, Sugi Y (1996) Epithelial-mesenchymal transformations in early avian heart development. Acta Anat 156:173–186
42. Medvinsky A, Dzierzac EA (1996) Definitive hematopoiesis is autonomously initiated by the AGM region. Cell 86:897–906
43. Medvinsky A, Samoylina NL, Müller AM, Dzierzak EA (1993) An early pre-liver intra-embryonic source of CFU-S in the developing mouse. Nature 364:64–66
44. Millauer B, Wizigmann-Voos S, Schnürch H, Martinez R, Møller NPH, 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
45. Mukai T, Terakado K, Reed CG (1997) *Bryozoa*. In: Harrison FW (ed) Microscopic anatomy of invertebrates. Vol. 13, Wiley-Liss, New York, pp. 45–206
46. Muller J, Yoshida T (1995) Interaction of murine peritoneal leukocytes and mesothelial cells: In vitro model system to survey cellular events on serosal membranes during inflammation. Clin Immunol Immunopathol 75:231–238
47. Muñoz-Chápuli R, Macías D, Ramos C, Fernández B, Sans-Coma V (1997) Development of the epicardium in the dogfish (*Scyliorhinus canicula*) Acta Zool (Stockholm) 78:39–46
48. Muñoz-Chápuli R, Macías D, Ramos C, Gallego A, Andrés V (1996) Development of the subepicardial mesenchyme and the early cardiac vessels in the dogfish (*Scyliorhinus canicula*). J Exp Zool 275:95–111
49. Murray PDF (1932) The development in vitro of the blood of the early chick embryo. Proc R Soc London 11:497–521
50. Newman PJ, Berndt MC, Gorski J, White GC, Lyman S, Paddock C, Muller WA (1990) PECAM-1 (CD31) cloning and relation to adhesion molecules of the immunoglobulin superfamily. Science 247:1219–1222
51. Noden D (1991) Development of craniofacial blood vessels. In: Feinberg RN, Sherer GK, Auerbach R (eds) The development of the vascular system. Karger, Basel, pp. 1–24
52. Olah I, Medgyes J, Glick B (1988) Origin of aortic cell clusters in the chicken embryo. Anat Rec 222:60–61
53. Page M (1989) Changing patterns of cytokeratins and vimentin in the early chick embryo. Development 105:97–107
54. Pardanaud L, Dieterlen-Lièvre F (1993) Emergence of endothelial and hemopoietic cells in the avian embryo. Anat Embryol 187:107–114
55. Pardanaud L, Dieterlen-Lièvre F (1993) Expression of *c-ets1* in early chick embryo mesoderm: relationship to the hemangioblastic lineage. Cell Adhesion Comm 1:151–160
56. Pardanaud L, Luton D, Prigent M, Bourcheix LM, Catala M, Dieterlen-Lièvre F (1996) Two distinct endothelial lineages in ontogeny, one of them related to hemopoiesis. Development 122:1363–1371

57. Pardanaud L, Yassine F, Dieterlen-Lièvre F (1989) Relationship between vasculogenesis, angiogenesis and haemopoiesis during avian ontogeny. Development 105:473–485
58. Pérez-Pomares JM, Macías D, García-Garrido L, Muñoz-Chápuli R (1997) Contribution of the primitive epicardium to the subepicardial mesenchyme in hamster and chick embryos. Dev Dyn 210:96–105
59. Pérez-Pomares JM, Macías D, García-Garrido L, Muñoz-Chápuli R (in press) Immunolocalization of the vascular endothelial growth factor receptor-2 in the subepicardial mesenchyme of hamster embryos. Identification of the coronary vessel precursors. Histochem J
60. Pérez-Pomares JM, Macías D, García-Garrido L, Muñoz-Chápuli R (1998) The origin of the subepicardial mesenchyme in the avian embryo: An immunohistochemical and quail-chick chimera study. Dev Biol 200:57–68
61. Risau W (1997) Mechanisms of angiogenesis. Nature 386: 671–674
62. Risau W, Flamme I (1995) Vasculogenesis. Annu Rev Cell Dev Biol 11:73–91
63. Satoh K, Prescott SM (1987) Culture of mesothelial cells from bovine pericardium and characterization of their arachidonate metabolism. Biochem Biophys Acta 930:283–296
64. Shalaby F, Rossant J, Yamaguchi TP, Gertsenstein M, Wu X-F, Breitman ML, Schuh AC (1995) Failure of blood-island formation and vasculogenesis in flk-1 deficient mice. Nature 376:62–66
65. Sugi Y, Markwald RR (1996) Formation and early morphogenesis of endocardial endothelial precursor cells and the role of endoderm. Dev Biol 175:1 66–83
66. 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
67. Topley N, Mackenzie RK, Williams JD (1996) Macrophages and mesothelial cells in bacterial peritonitis. Immunobiology 195:563–573
68. Turbeville JM (1991) *Nemertinea*. In: Harrison FW (ed) Microscopic Anatomy of Invertebrates. Vol. 3, Wiley-Liss, New York, pp. 285–328
69. Van Hinsberg VWM, Kooistra T, Scheffer MA, van Bockel JH, van Muijen GNP (1990) Characterization and fibrinolytic properties of human omental tissue mesothelial cells. Comparison with endothelial cells. Blood 75:1490–1497
70. Van Muijen GN, Ruiter DJ, Warnaar SO (1987) Coexpression of intermediate filament polypeptides in human fetal and adult tissues. Lab Invest 57:359–369
71. Vanden Bossche JP, Jangoux M (1976) Epithelial origin of starfish coelomocytes. Nature 261:227–228
72. Vandenbunder B, Pardanaud L, Jaffredo T, Mirabel MA, Stehelin D (1989) Complementary patterns of expression of c-ets 1, c-myb and c-myc in the blood-forming system of the chick embryo. Development 107:265–274
73. Viebahn C, Lane EB, Ramaekers CS (1987) The mesonephric (Wolffian) and paramesonephric (Müllerian) ducts of golden hamsters express different intermediate-filament proteins during development. Differentiation 34:175–188
74. Viebahn C, Lane EB, Ramaekers CS (1988) Keratin and vimentin expression in early organogenesis of the rabbit embryo. Cell Tissue Res 253:553–562
75. Visvader J, Fujiwara Y, Orkin SH (1988) Unsuspected role for the T-cell leukemia protein SCL/tal-1 in vascular development. Genes Dev 12:473–479
76. Young PE, Baumhueter S, Lasky LA (1995) The sialomucin CD34 is expressed on hematopoietic cells and blood vessels during murine development. Blood 85:96–105