Review

Pericytes in the microvasculature

Karen K. Hirschi, Patricia A. D'Amore *

Laboratory for Surgical Research, Children's Hospital, Department of Pathology, Harvard Medical School, Enders 1061, 300 Longwood Avenue, Boston, MA 02115, USA

Abstract

Pericytes, also known as Rouget cells or mural cells, are associated abluminally with all vascular capillaries and post-capillary venules. Differences in pericyte morphology and distribution among vascular beds suggest tissue-specific functions. Based on their location and their complement of muscle cytoskeletal proteins, pericytes have been proposed to play a role in the regulation of blood flow. In vitro studies demonstrating the contractile ability of pericytes support this concept. Pericytes have also been suggested to be oligopotential and have been reported to differentiate into adipocytes, osteoblasts and phagocytes. The mechanisms involved in vessel formation have yet to be elucidated but observations indicate that the primordial endothelium can recruit undifferentiated mesenchymal cells and direct their differentiation into pericytes in microvessels, and smooth muscle cells in large vessels. Communication between endothelial cells and pericytes, or their precursors, may take many forms. Soluble factors such as platelet-derived growth factor and transforming growth factors-beta are likely to be involved. In addition, physical contact mediated by cell adhesion molecules, integrins and gap junctions appear to contribute to the control of vascular growth and function. Development of culture methods has allowed some functions of pericytes to be directly examined. Co-culture of pericytes with endothelial cells leads to the activation of transforming growth factor-beta, which in turn influences the growth and differentiation of the vascular cells. Finally, the pericyte has been implicated in the development of a variety of pathologies including hypertension, multiple sclerosis, diabetic microangiopathy and tumor vascularization.

Keywords: Vasculogenesis; Angiogenesis; Pericytes; Blood flow; Growth factors; Gap junctions

1. Pericyte characteristics

1.1.1. Structure and distribution

Perivascular cells in close association with capillaries were first noted over 100 years ago by Eberth [1] and Rouget [2,3]. Zimmermann [4] introduced the term "pericyte" to describe these cells that are: found adjacent to capillaries in a variety of tissues in many species, continuous with vascular smooth muscle cells of arteries and veins, and distinctively shaped with many cytoplasmic processes that encircle capillaries.

Each pericyte possesses a cell body with a prominent nucleus and a small amount of surrounding cytoplasm [5–7]. Protruding from the cell body are long processes which parallel the long axis of the capillary and taper to smaller processes which encircle the capillary wall. Peri-cytes are embedded within a basement membrane which surrounds the capillary tubes. In vitro evidence suggests that both endothelial cells (EC) and pericytes contribute to the formation of the basement membrane [8]. Their processes penetrate the basement membrane to directly contact the underlying endothelium and, in a reciprocal manner, endothelial processes penetrate into the pericytes [9]. Early studies by Zimmermann [4] revealed the presence of ~ 90 processes with a width of 0.3–0.8 μm per 100 μm of capillary length. Interestingly, the distance between cell bodies is ~ 70 μm in the retina and 50 μm in the brain, suggesting an interdigitation of processes among neighboring pericytes. In fact, a single pericyte can extend processes to more than one capillary within the microcirculation [7,10,11]. The physiological significance of this is currently unknown, as is whether the number or size of

---

* Corresponding author. Tel. (+ 1-617) 355-8377; Fax (+ 1-617) 355-7043; E-Mail: damore_p@A1.tch.harvard.edu

Time for primary review 15 days

pericyte processes varies among different tissues and species.

The number of pericytes per se, however, varies significantly in different tissues and among different sized vessels. On either side of a capillary bed, there is a continuum of cells from true pericytes surrounding capillaries, to intermediate cells resembling both smooth muscle cells (SMC) and pericytes at the interface between arteriolar or venular capillaries and arterioles or venules, to true SMC surrounding terminal arterioles, venules and larger vessels [12,13]. Furthermore, there are structural differences among pericytes on the arterial and venous sides of capillary beds. In general, pericytes are more numerous and have more extensive processes on venous capillaries and post-capillary venules [14]. Also, pericytes on larger venules, compared to those of venous capillaries, tend to be longer and exhibit more contact with the endothelium [12].

The differences in distribution and structure among pericytes suggest that they may have vessel- or tissue-specific roles. Hence, pericytes have a variety of proposed functions including: regulation of capillary blood flow; as multipotent mesenchymal cells and specific precursors to vascular SMC; phagocytosis; and regulation of new capillary growth.

1.1.2. Regulation of flow

The proposed role of pericytes in capillary blood flow is based predominantly on morphological and ultrastructural observations. Pericytes exhibit a number of characteristics consistent with muscle cell activity and their location in capillaries would enable them to regulate the contraction of the underlying endothelium. The presence of actin in pericytes has been demonstrated on the abluminal cytoplasm of foot processes [15,16]. Furthermore, the adhesive protein fibronectin has been localized to the junctional regions between pericyte processes and EC [17] and may anchor the pericyte during the transfer of contractile force.

More recent immunocytochemical and biochemical studies provide further evidence for contractile machinery within pericytes and indicate that they may function in a manner analogous to vascular SMC. Herman and his coworkers [18,19] and Skalli et al. [20] used isoform-specific antibodies to demonstrate smooth muscle actin in microvascular pericytes. Nehls and colleagues [21] reported that only pericytes of pre-capillary and post-capillary venules of the bovine retinal microcirculation expressed muscle-specific actin. Herman et al. and Skalli et al. did not focus on the particular location within the vascular bed of the pericytes that they were observing. It is not clear if there is truly an all-or-none distribution of muscle actin within pericytes of the microvasculature or if the differences are due to detection sensitivity of the methods used. The level of muscle actin detected in pericytes, in general, was intermediate between that found in vascular SMC of arteries and arterioles and that in endothelial cells.

There is, however, a suggestion of pericyte heterogene-

ity based on their expression of α-SM actin. Nehls and Drenckhahn [21] found that only the pericytes of pre- and post-capillary microvessels of bovine retina expressed α-SM actin; whereas, midcapillaries had no detectable levels. In contrast, others have reported α-SM actin and myosin in the brain microvasculature [22–24]. A systematic study of α-SM actin mRNA and protein in brain revealed mRNA in freshly isolated microvessels (containing both EC and pericytes) and in pericytes in vitro, but not in cultured EC [25]. Immunohistochemical localization of the α-SM actin protein revealed staining in precapillary arterioles but not in capillary EC or pericytes. These authors hypothesize that only the α-SM actin-expressing capillaries participate in blood flow control and the mid-capillaries serve a different function.

The concept that pericyte expression of α-SM actin differs between the pericytes of the post-capillary microvessels and those of true capillaries is intriguing. However, since the vasculature otherwise appears to have gradients of both expression and function, it seems more likely that the pericytes of true capillaries may have less α-SM actin than the pericytes of their neighbors and not lack α-SM actin altogether. The limits of sensitivity of immunohistochemical analysis may prevent observation of reduced α-SM actin levels. The fact that many have reported α-SM actin in pericytes both in vivo and in vitro lends some credence to this suggestion.

Whether pericyte contraction is a universal or restricted function remains to be determined, but these cells, in general, exhibit other features of muscle-like cells. Tropomyosin and muscle myosin isoforms are expressed in a pattern similar to that of α-SM actin with levels in pericytes somewhat lower than that of vascular SMC [26,27]. Joyce and coworkers [28] also demonstrated the presence in pericytes of cyclic GMP-dependent protein kinase, which is involved in the regulation of smooth muscle cell contraction.

Although direct evidence of pericyte contraction in vivo is limited, Tilton and his coworkers [9], using ultrastructural morphometric techniques, demonstrated the compression of endothelial cell membranes by apposing pericytes and reported a pericyte contractile response to vasoactive agents in skeletal muscle. Interestingly, Il-2 treatment which led to increased albumin permeability in rat muscle, was found to alter the distribution of pericytes from a distribution that was more random to a pattern of localization around junctions [29]. It should also be noted in the context of this discussion of "contractility" at the level of the capillary that extremely small changes in the internal diameter of a capillary would lead to reduced blood flow. The diameter of a true capillary is just sufficient for the passage of a red blood cell. Very subtle narrowing of the lumen, which would presumably result from a "contraction" of the pericyte, may be below the level of resolution of our morphological methods yet functionally very effective at altering blood flow.

1.1.3. In vitro studies

Using an in vitro model, Kelley and her co-workers [30] reported that retinal pericytes cultured on a collagen lattice decrease the surface area of the lattice over several days. Furthermore, the contractile activity of pericytes cultured on a silicon rubber substrate is modulated by vasoactive agents [31]. Das et al. [32] also demonstrated the contraction of pericytes in culture by permeabilizing the cells and measuring a dose-dependent response to ATP. In these same experiments, endothelial and epithelial cells did not demonstrate contractile activity.

Additional evidence for a role of pericytes in the regulation of capillary flow comes from the identification of known regulators of vessel "tone". Whereas EC are well known to produce the vasoconstrictor endothelin 1 (reviewed in [33]), pericytes have been shown to express endothelin receptors [34]. Similarly, EC produce the potent vasodilator nitric oxide (NO) which leads to the relaxation of SMC via a cGMP-dependent mechanism (reviewed in [35]). Since pericytes are similar to SMC in many aspects of their contractile machinery, it is reasonable to suspect that EC-derived NO leads to comparable effects in the capillary. Evidence for this has been provided using in vitro systems where sodium nitroprusside was shown to lead to relaxation of pre-contracted pericytes [31,36].

1.1.4. Pericytes as SMC precursors

The marked similarity to vascular SMC has led to the theory that pericytes are specific precursors of SMC [13]. A study of mesenteric capillary growth in rats [37] suggests that fibroblasts transform into capillary pericytes which, in turn, become vascular smooth muscle cells. Hence, as capillaries are remodeled into larger vessels to meet an increased functional demand, pericytes are further differentiated to become true SMC, as needed.

1.1.5. Pericytes as pluripotent cells

Pericytes are also thought to be multipotent, capable of becoming adipocytes, osteoblasts and phagocytes. Richardson et al. [38] reported the conversion of capillary pericytes to immature adipocytes in the inguinal fat pad of rats in response to thermal injury. They found an increase in rough endoplasmic reticulum in the pericytes, movement away from capillary basement membrane and subsequent differentiation into immature adipocytes. These data are consistent with a previous suggestion that the adipocyte precursor cell is a perivascular cell, associated with the reticuloendothelial system [39].

A role for pericytes as osteoblast progenitors has also been proposed. In ultrastructural studies of electrically induced osteogenesis in rabbit, a close spatial relationship between microvessels, polymorphic osteoblast precursor cells, osteoblasts and bone was noted [40]. Subsequent studies [41] demonstrated that pericytes in vitro form colonies that incorporate calcium phosphate, produce an extracellular matrix (ECM) rich in alkaline phosphatase

and collagen, and synthesize large amounts of osteocalcin. Furthermore, in a model of bone formation using rat femur [42], pericytes in preexisting microvessels were labeled with Monastral blue and were shown to migrate toward, and become incorporated into, newly formed bone tissue as osteoblasts.

Many investigators [43–46] have also suggested that brain pericytes are microglial cells and, hence, the precursors to brain macrophages. In fact, Maxwell and Kruger [43] reported that following irradiation of the brain, pericytes undergo mitosis and penetrate the brain parenchyma to become microglia. Phagocytic activity of pericytes was first demonstrated using electron microscopic techniques. Majno et al. [47] observed that following histamine treatment, pericytes of venules gradually take up carbon over the course of several months. In further support of the theory that pericytes act as phagocytic cells, systemic injection of protein tracers into immature mice lead to an accumulation of tracer in the pericytes of the brain and spinal cord [48]. Similarly, exogenous administration of trypan blue and horseradish peroxidase lead to uptake by cerebral pericytes [49]. Markov and Dimova [50] found that, following lead poisoning, brain pericytes become hypertrophic, increasing their cell volume. Pericytes are also thought to be structurally homologous to mesangial cells in the glomerulus [51], which demonstrate significant phagocytic properties [52].

1.1.6. Pericytes and capillary growth control

Finally, pericytes may play a regulatory role in the control of capillary growth. For example, during wound healing, it is thought that pericytes from preexisting microvessels, or newly derived pericytes, come into contact with endothelial cells that are forming new vessels and exert an inhibitory effect on EC proliferation [53]. Evidence to support these concepts about the regulatory interactions between endothelial cells and pericytes comes from in vitro studies and will be discussed in detail in subsequent sections.

2. Vessel formation

Blood vessels are among the first organs to develop during embryogenesis and are derived entirely from the mesoderm [54,55]. Blood vessels, in general, are composed of distinct cell layers, although their exact cellular composition varies with vessel size, reflecting in part, differences in vascular function. As previously mentioned, small blood vessels such as capillaries are composed of EC surrounded by a basal lamina and a single layer of pericytes. The outermost layer of capillaries, or adventitia, is a thin layer of connective tissue that is continuous with that of the surrounding tissue.

Large blood vessels are comprised of three layers: the intima, media and adventitia. The intima, the innermost

layer, is made up of one cell type — the endothelium — which lines the lumenal surface. Intimal SMC can also be found but their frequency appears to be a function of species, age and pathology. The media is composed of multiple layers of SMC, and is separated from the intima by an internal elastic lamina. The outermost adventitial layer consists of loose connective tissue and contains smaller blood vessels and nerves.

2.1.1. Pericyte recruitment in vessel formation

Blood vessel assembly during embryogenesis, a process known as vasculogenesis, begins with clustering of primitive vascular cells or hemangioblasts, into blood islands [56]. These blood islands consist of endothelium and primitive blood cells and give rise to tube-like endothelial structures which define the pattern of the vasculature [57]. Branching and remodeling of such structures, a process known as angiogenesis, leads to the formation of a primitive vascular network.

Embryonic data suggest that the initial endothelial tubes may be responsible for subsequent development of vessel layers; that is, the presumptive intimal endothelium may function to recruit and organize mural cells (pericytes in the microvasculature; smooth muscle cells in large vessels). As the endothelial tubes invade organ primordia, they become surrounded by locally-derived mesenchymal cells (i.e. precursors of mural cells; adventitial fibroblasts) [58]. Hence, the primordia themselves contribute the mural cell layer(s) to the developing vessels, which may result in tissue-specific functional and regulatory properties of pericytes, as well as SMC. This is in contrast to EC which are thought to be of uniform origin [59].

The exact mechanism(s) by which EC recruit pericytes or SMC during vessel formation is unknown. Since there appears to be a tight control between the number of EC and mural cells at any given point in the vascular tree, it is likely that multiple sites of control exist (Fig. 1). Potential regulators include soluble factors that act in an autocrine and/or paracrine manner, mechanical forces secondary to blood flow and blood pressure, as well as homotypic (EC–EC, mural cell–mural cell) and heterotypic (EC–mural cell) cell contacts.

It is well known that EC and mural cells communicate, and thereby influence each other's behavior, via bi-directional extracellular exchange of soluble mediators. EC are a source of diffusible factors which regulate mural cell, and possibly mural cell-precursor, proliferation and migration. Hence, EC–mural cell precursor interactions mediated by soluble effectors may play an important role in blood vessel morphogenesis, as well as in a variety of vascular diseases which are discussed in Section 6 below.

2.1.2. Soluble effectors of pericyte recruitment by EC

Among the diffusible soluble factors synthesized and secreted by EC, are a variety of polypeptide growth factors, including platelet-derived growth factor (PDGF) [60]
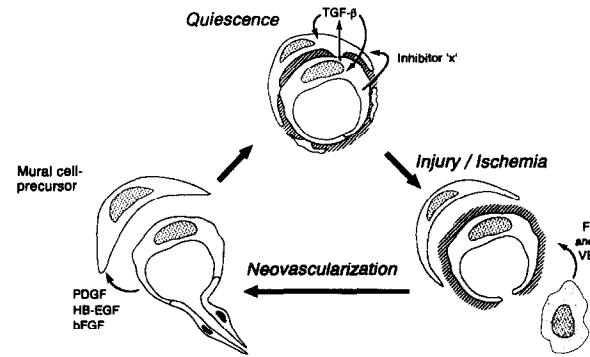

Fig. 1. Cell–cell interactions in forming vessels. EC recruit mesenchymal cells via the elaboration of soluble factors such as platelet-derived growth factor (PDGF), heparin-binding epidermal growth factor (HB-EGF) or basic fibroblast growth factor (bFGF). The mural cell precursors migrate to the EC where they make contact. The heterotypic interactions lead to the activation of transforming growth factor-beta 1 (TGF-β1), which induces the mesenchymal cells to express SMC/pericyte markers and inhibits EC proliferation. Alterations in the microenvironment brought about by injury and/or ischemia disrupt this balance leading to local increases in growth stimulators such as FGF and vascular endothelial growth factor (VEGF). These changes result in the cell proliferation that characterizes normal wound healing and a variety of pathologies.

and basic fibroblast growth factor (bFGF) [61], which appear to function in a paracrine fashion to promote mural cell proliferation and/or migration. PDGF is a dimeric molecule consisting of homo- and heterotypic dimers of two subunits, PDGF-A and PDGF-B, namely PDGF-AA, PDGF-BB and PDGF-AB. Although all three forms of PDGF are functional, the response of a particular cell is dependent on its specific complement of receptors. The PDGF receptor consists of two subunits, alpha and beta. The alpha subunit binds both A and B chains, whereas the beta subunit binds only the B chain [62].

The spatial distribution of active PDGF ligand and receptor genes in both early and late stages of human placental development was analyzed by Holmgren et al. [63] and their observations suggest a role for PDGF in vessel formation. They found that the microcapillary EC co-express PDGF-B and PDGF-β-receptor genes, indicating that EC proliferation may be stimulated in an autocrine fashion by this molecule during placental angiogenesis. Furthermore, EC of larger, developing blood vessels maintain high PDGF-B expression, but have no detectable PDGF-β-receptor mRNA. In contrast, PDGF-β-receptor mRNA is readily detectable in fibroblast-like cells and SMC in the surrounding intima of intermediate and macro blood vessels. These data suggest that EC-derived PDGF-B may have a paracrine effect on mural cell-precursor migration and proliferation during vessel development. In support of this theory, we have demonstrated (Gabriels and D'Amore, manuscript submitted), using an in vitro co-culture system, that SMC migrate directionally toward EC. Neutralizing antibodies to PDGF-B inhibit the EC-stimulated migration of SMC, suggesting that PDGF may be involved in the EC–mural cell precursor interactions nec-

essary for vessels to form (see Fig. 1).

bFGF, a member of the family of heparin-binding mitogens, is a single polypeptide chain with an isoelectric point of 9.6 that exists in several isoforms ranging in molecular weights from 18 to 25 kD. EC have been reported to synthesize but not secrete bFGF; however, there are reports that bFGF can be associated with the ECM [64,65]. The release of bFGF from the cell cytoplasm may occur during cell injury or death [66], whereas the release of stored bFGF from the ECM may occur via displacement with heparin or by degradation by heparinases [67]. bFGF is angiogenic, as well as mitogenic [68] and chemotactic [69] for SMC. Thus, although its mechanism of release remains unclear, it is also a candidate for involvement in SMC- or pericyte-precursor recruitment by EC.

Another member of the family of heparin-binding mitogens is heparin-binding epidermal growth factor (HB-EGF). Initially, it was purified from macrophages [70]. HB-EGF stimulates the migration of SMC [71] and pericyte proliferation (D'Amore and Klagsbrun, unpublished data), but not EC. HB-EGF has 40% homology with EGF, but possesses an N-terminal extension, which confers heparin affinity. Other sites of HB-EGF synthesis have not been identified, but its production by vascular EC [72] makes it a candidate-effector for EC recruitment of mural cells.

Connective tissue growth factor (CTGF) is a 38-kD cysteine-rich secreted protein that is immunologically related to PDGF and 45% homologous to the SRC-induced immediate early gene product CEF-10 [73]. CTGF is a mitogen and chemoattractant for mesenchymal cells. It was identified in conditioned media (CM) derived from human umbilical vein EC. Hence, it may also be an effector in EC recruitment of mural cell precursors during vessel formation.

3. Pericyte interactions with endothelial cells

Soluble regulators, as well as cell–cell contacts, may also play a role in the differentiation of mural cell-precursors into pericytes or SMC (Fig. 2). Once the pericyte precursors have been recruited to the EC, the two cell types make contact through discontinuities in the vessel basement membrane [74]. Similarly, myoendothelial junctions in large vessels occur through fenestrations in the internal elastic lamina [75]. These EC–mural cell junctional complexes are thought to involve cell-adhesion molecules (CAMs), substrate adherence molecules (SAMs), the ECM itself, and gap junctions [76]. Although few data exist, any of these factors, as well as soluble regulators, may contribute to the differentiation of pericytes and SMC.

Although the precise nature of the contact is unknown, it is clear from previous studies by ourselves and others that cell–cell contact is necessary for the functioning of at least one growth factor — transforming growth factor type β1 [77]. Both EC and mural cells grown separately were
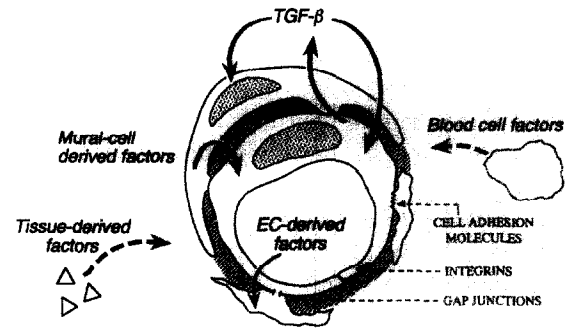
Fig. 2. Cell–cell interactions in the microvasculature. EC and pericytes of microvessels communications via a variety of mechanisms including cell adhesion molecules, integrins, gap junctions, and soluble factors derived from tissues, blood cells and the cells themselves.

demonstrated to produce a latent form of TGF-β1, which is activated upon EC–mural cell contact (see below for details). This phenomenon takes on particular relevance in light of evidence that TGF-β induces the expression of α-SM actin (a marker for SMC) expression in myofibroblasts [78] as well as in pericytes [79]. Furthermore, in mice that lack TGF-β1, there is insufficient vascular development secondary to suppression of EC differentiation [80]. Hence, TGF-β1 may influence both EC and mural cells during vessel formation.

The role of EC–mural cell contacts is almost certainly not limited to vessel formation. The same types of cell–cell interactions may be vital to the maintenance and functioning of the quiescent vessel (see Fig. 2). In the adult vessel, EC and pericytes in vivo are likely to continue to synthesize and secrete soluble factors which exert bi-directional effects on growth and differentiation (reviewed in [81]). For instance, the conditioned media from confluent EC inhibit the growth of mural cells in vitro [82], emphasizing the intercellular growth regulatory capacity in this system.

3.1.1. Gap junctions

Gap junctions are aggregates of intercellular channels that connect the cytoplasms of adjoining cells and allow the passage of small molecules (< 1000 kD), ions and nutrients. Although gap junctions between EC and mural cells have been detected in vitro [83,84], their exact function in vascular cells is unknown. Gap junctions have been implicated in cellular interactions during growth and development in a variety of systems (for review, see [85,86]) where they are thought to be conduits for growth-modulating signals. This may also be the case in the vasculature.

Data regarding junctional communication in vivo are mixed. Segal and Beny [87] injected Lucifer yellow into cells of the hamster cheek pouch arterioles. When EC were injected, the dye was transferred to numerous neighboring EC. On the other hand, SMC injected with dye did not transfer to other SMC and neither cell type showed evidence of transfer to the other. More recent data using the same model suggest that whereas EC are well-coupled, there is less effective transfer of dye through SMC junc-

tions [88]. Junctions between EC and SMC revealed a dramatic polarity; dyes transferred from EC to SMC but there was virtually no transfer in the reverse direction. The differing results may be due to different dyes that were used to assess gap junctional communication. Studies of dyes indicate that transfer among various gap junction channels depends on their protein composition [89]. The latter study employed biotin which the authors comment "is superior to Lucifer yellow in tracing gap junctions". Although equivalent studies have not been conducted in the microvasculature, the similarities between large and small vessel EC, and SMC and pericytes, suggest that junctional communication may also occur in capillaries. The exact role of gap junctional intercellular communication between these cells is unknown, but it is thought to play a role in growth control and blood flow. Also unknown is the exact connexin (Cx) composition of the EC and mural cells in vivo.

The endothelium has been shown to express Cx40 [90–92] and Cx37 [93] in vivo. Contrary to the findings of Bruzzone and coworkers [90], Cx43 was also detected in arteriolar EC [92]. Some investigators detected only Cx43 in SMC [90,94], whereas others also found Cx40 [92]. Furthermore, the A7r5 aortic SMC line was shown to express both Cx43 and Cx40 [95].

It will be of interest to determine the Cx composition of various vessels within the same animal and whether there are cross-species differences. The patterns of Cx expression may change as a function of vessel size, physiological role and species. If EC in vivo express only Cx40 and Cx37 as some researchers believe, and mural cells express only Cx43, EC and SMC would be capable of communicating through only heterotypic channels of Cx43 and Cx37, as functional heterotypic channels do not form between Cx43 and Cx40 [96]. However, if EC and mural cells express the same Cx type, they would then be able to communicate through homotypic channels (i.e. Cx43–Cx43 and Cx40–Cx40). This may be relevant because it is possible that different channel types and partners represent differing gap junction functions.

3.1.2. Cell adhesion molecules

Regardless of the Cx composition of the gap junction channels in EC and mural cells, it is known from previous studies that gap junction membrane-insertion and function, in general, is dependent on the co-expression of appropriate cell adhesion molecules [97,98]. The exact CAMs which interact with gap junctions in the vasculature have not been identified. However, the identity and independent roles of many CAMs in vessels have been examined.

EC are known to express the Ca^{2+}-sensitive adhesion molecules VE-cadherin (or cadherin-5) [99], P-cadherin [100], E-cadherin (specifically in brain capillaries — [101]) and N-cadherin [102]. Mural cells express N-cadherin, as well, which has been proposed to mediate EC–SMC interactions in vitro and in vivo [103].

EC and mural cells also express CAMs that mediate interactions amongst themselves or with other cell types. For example, pericytes have been reported to express V-CAM, which was increased by treatment with TNF-α [104]. Similarly, EC express PECAM-1 [105] and P- and E-selectin (reviewed in [106]) which are thought to mediate EC–platelet, EC–leukocyte and/or EC–EC interactions. Though there has been no definitive demonstration regarding the function of CAMs on pericytes, involvement of these adhesion molecules in inflammatory responses is a likely role [104,107].

3.1.3. Integrins

Integrins are also expressed in both EC and mural cells. These molecules are cell surface adhesion receptors that anchor cells to the ECM and cytoskeleton, as well as mediate transmembrane signaling (reviewed in [108,109]). Thus, the cells of the vasculature have a complex environment in which multiple forms of cell–cell signaling occur in concert.

4. Tissue culture

The isolation and culture of microvascular pericytes has been accomplished and usually involves a combination of mechanical disruption and enzymatic digestion (for details see D'Amore [110]). Because of the tight attachment between pericytes and capillary endothelial cells, it is difficult to isolate a significant number of pericytes totally free of capillary endothelium. However, even though primary cultures of pericytes in the first days following the isolation procedure may contain numerous endothelial cells, the cultures become more homogenous with time. Certain culture conditions appear to contribute to the fact that pericyte growth predominates over that of the EC. First, growing endothelial cells synthesize platelet-derived growth factor A and B chains [111–113] as well as heparin-binding EGF (HB-EGF) [72] which are potent stimulators of pericyte proliferation ([68] and Klagsbrun and D'Amore, unpublished data). Although pericytes make endothelial mitogens, the EC-derived pericyte growth factors appear to dominate. Second, the primary cultures are established on uncoated tissue culture plastic whereas capillary EC have historically been noted to prefer coatings with some matrix component such as gelatin or fibronectin [114]. Third, and perhaps most importantly, endothelial cell–pericyte co-cultures lead to the inhibition of endothelial growth.

During our early efforts to establish primary cultures of microvascular cells from the bovine retina, we noted small colonies of EC, interspersed with isolated pericytes. With time, the pericyte number increased and endothelial cell colonies appeared to cease growing and occasionally detach from the substrate. Subsequently, we showed endothelial cell–pericyte co-culture leads to the activation of

transforming growth factor type β1 (TGF-β), a potent inhibitor of endothelial cell proliferation [115] (see below for more details). Although the proliferation of pericytes is also inhibited by TGF-β [68], we suspect that the level of pericyte mitogens derived from the growing EC is sufficient to overcome the inhibitory actions of the TGF-β. Thus, the culture conditions under which primary cultures of pericytes are established appears to favor the proliferation of pericytes over EC and thus permits the culture of pericytes for cell biological, biochemical, and molecular biological studies described below.

5. Co-culture models

Interesting information regarding the possible functions of pericytes has been derived by studying homogenous cultures of pericytes. These include their ability to make a variety of ECM components [8], their contractile activity [30], and their response to a variety of growth stimulators and inhibitors [23,68]. The list of products made by pericytes is expanding as quickly as the number of investigators that culture the cells. For example, pericytes have been shown to synthesize the ECM proteins fibronectin and laminin [8], collagen [116] and glycosaminoglycans [117].

However, pericytes exist in very close association with microvascular EC in vivo. Thus, their physiologic functions are best examined in the context of their interactions with microvascular endothelium. For this reason, we and others have developed a variety of co-culture models in which to examine the intercellular communication between pericytes and microvascular endothelium. There have been a limited number of studies of cell–cell interactions in the microvasculature using microvascular EC in co-culture with pericytes. The most extensive investigations of co-culture effects have focused on growth control. Co-culture of pericytes with EC inhibits the growth [118] and migration [119] of the EC. The inhibition of the EC is dependent on contact between pericytes and is due to the activation of TGF-β1 [77,119]. Although the precise details of the interaction which lead to TGF-β activation have yet to be clarified, the ability of agents that interfere with the plasminogen activator system to prevent the activation suggests a role for plasmin [119]. In addition, the inclusion of mannose 6-phosphate in co-cultures of EC and SMC also blocked the generation of inhibitory activity [120]. The inhibitory effect of the mannose 6-phosphate is thought to be due to its blocking the binding of latent TGF-β1 to the surface of the SMC, where it would presumably become a more effective substrate for the plasmin.

It is clear that the interactions between EC and pericytes similarly influence pericyte proliferation. Conditioned media collected from EC vary in their effects on mural cells, depending on the density of the conditioning EC [82]. Media collected from sparse EC either have no effect or

are mildly stimulatory for SMC or pericyte growth whereas media conditioned by post confluent EC are strongly inhibitory. Similar inhibitory activity is observed when EC and SMC are cultured on opposite sides of a porous membrane [121]. These results are consistent with the nature of the interactions that might be expected between the two cell types. Growing EC, which characterize development or certain pathologies, would be expected to induce proliferation of mural cells to associate with newly forming vessels. In contrast, the mural cells of mature vessels are quiescent and it is reasonable to suspect that EC play a role in maintaining this suppressed growth state.

5.1.1. In vitro angiogenesis

EC in culture have been shown to form tube-like capillary structures under a variety of conditions [122–124]. Because of their abluminal location in vivo some investigators have postulated that the pericyte might stimulate this process. However, co-cultivation of EC with either pericytes or SMC did not increase the number of capillary-like tubes formed in a fibrin gel [125]. We believe that rather than increase the rate and/or number of tubes formed in culture, that pericytes might "stabilize" these structures by altering the EC phenotype to reflect a more differentiated state. At present, this is a somewhat difficult hypothesis to test as the differentiated phenotype of the EC has not been well-defined. However, a suggestion that tube formation is itself a more differentiated state was provided in studies showing that the synthesis of PDGF-B is down-regulated in capillary EC in tubes versus their monolayer counterparts [126]. Studies on the effects of pericyte co-culture on PDGF expression by EC in tubes or monolayers have not been reported.

5.1.2. Effects on EC metabolism

Very few studies have been conducted to examine the effects of pericytes on EC functions other than growth and migration. In one study pericytes (but not fibroblasts) were shown to significantly stimulate prostacyclin production by EC as well as protect the EC against lipid peroxide-induced damage [127].

Studies of reciprocal interactions (e.g. EC effects on pericyte metabolic function) are lacking. There are some reports of EC influences on SMC function (for review see [128]). For instance, EC were shown to alter low density lipoprotein metabolism by SMC [129] by increasing the number of high affinity receptors and the rate of LDL endocytosis and degradation. Because of the many similarities between pericytes and SMC, it is reasonable to suspect that similar effects might be observed in EC–pericytes co-cultures. However, given their different locations within the vasculature it is also reasonable to suspect that there will be functional differences between pericytes and SMC.

5.1.3. Control of pericyte growth

Virtually nothing is known about the regulation of pericyte growth in vivo. Above we outline a hypothesis suggesting that pericyte growth during vessel formation may be positively regulated by PDGF-B. This is supported by the observation of PDGF-β receptors on pericytes under conditions of active growth including healing wounds and tumors [130]. Results of in vitro analysis of pericyte response to PDGF isoforms are consistent with these observations. We found that pericytes in vitro respond maximally to PDGF-BB, with virtually no proliferation induced by PDGF-AA and very little by PDGF-AB [68]. Interestingly, SMC were stimulated to a greater extent by PDGF-AA and PDGF-AB. These data suggest that pericytes may possess a lower level of PDGF-α receptor. However, in vitro data on the PDGF-α receptor have not been reported and these findings may also be the result of changes in the cell resulting from tissue culture.

Other polypeptide growth factors which have been shown to be mitogenic for pericytes include: basic and acid fibroblast growth factors [68], heparin-binding epidermal growth factor (Klagsbrun and D'Amore, unpublished results) and endothelin [131]. Pericyte growth has been shown to be inhibited by transforming growth factor-β1 [68], and by heparin [132]. Furthermore, pericyte proliferation has been documented to be modulated by various ECM components. Matrix prepared from EC by deoxycholate treatment induced significant pericyte proliferation (between 2.5- and 4-fold) [132]. In another series of studies EC matrices prepared by alkali or Triton X-110 inhibited pericyte proliferation [133]. The differences between these results are most likely due to modifications resulting from the matrix preparation. For instance, latent TGF-β associated with the ECM may have been activated in the latter study but not in the former. Purified matrix components such as type IV collagen and fibronectin led to moderate but reproducible pericyte growth (20–70%).

6. Pericytes and pathology

The role of pericytes in disease processes has been attracting an increasing amount of attention [134,135]. The pericyte has been implicated in hypertension. Comparing distribution of pericytes in the Wistar-Kyoto rat to that in the spontaneously hypertensive rat (SHR), Herman and Jacobson [136] found four times the number of α-smooth muscle actin-positive pericytes in the SHR brains as compared to their normal counterparts. The difference was most dramatic in the tegmentum of the brainstem where virtually every capillary cross-section had an associated pericyte. Although these observations do not distinguish between a cause-and-effect relationship between mural cell proliferation and increased resistance, it is intriguing to postulate that EC dysfunction in the SHR animals mediates this effect. From that viewpoint these changes are similar

to that seen in the microvasculature in fetal pulmonary distress and in larger vessels during atherogenesis.

The microvasculature has been implicated in the pathogenesis of multiple sclerosis: T-cell adhesion to brain microvessels and migration into the brain are key events. Immunohistochemical analysis of brain section from patients with multiple sclerosis demonstrated VCAM-1 on both EC and pericytes and T-cell adherence to both cell types, a process increased by TNF-α treatment [104]. It was therefore suggested that not only the EC, but the pericytes, may play an important role in regulating T-cell infiltration into the central nervous system.

6.1.1. Diabetic microangiopathy

Although alterations in pericytes are described in a variety of tissues from diabetic individuals including skeletal muscle [137] and nervous tissue [138], the changes that occur in retinal capillaries are by far the best documented. Perhaps this is because the consequences of microvascular disease in the eye leads to such devastating complications. The loss of pericytes in retinal microvessels is one of the earliest changes that take place in retinal capillaries. Although many believe the "pericyte drop-out" (as it is called) to be the result of glucose damage, the exact mechanism which underlies their degeneration has not been definitively elucidated. Pericyte loss has been demonstrated in a number of animal models of diabetes including galactosemic rat and dog, stretozotocin-induced diabetic rat and rats with genetically-induced diabetes (described in [139]). Support for the concept that pericyte degeneration is secondary to glucose damage comes from studies in which diabetic-like retinal vessel changes in a galactosemic rat model, including pericyte loss, microaneurysms and acellularity, were prevented by oral treatment with Tolrestat, an aldose reductase inhibitor [140,141].

The physiologic consequences of pericyte loss have yet to be definitely elucidated. However, ultrastructural analyses of retinal microaneurysms reveal a consistent absence of pericytes, suggesting that loss of vessel integrity due to loss of pericytes may render vessels vulnerable to aneurysms. The correlation between the absence of pericytes and the retinal neovascularization in the retinopathy of prematurity and proliferative diabetic retinopathy led to the hypothesis that pericytes may have a suppressive influence in capillary growth [142]. Consistent with this concept was the ultrastructural observation that pericyte association with the developing capillary marked the cessation of vessel growth and the deposition of a basement membrane [53]. These findings led these investigators to make the following statement. "The incorporation of pericytes within the basement membrane of proliferating capillaries is proposed as the mechanism for inhibition of capillary proliferation". Although the hypothesis that pericyte control of capillary proliferation has not been tested directly in vivo, tissue culture studies in which EC have been cultured with pericytes (see above for details) indicate that there are

interactions between the two cells that may alter capillary growth.

6.1.2. Pericytes in tumors

Immunohistochemical studies have revealed the presence of many α-SMC actin-positive cells in the microvasculature of glioblastoma multiforma [143]. The identity of the α-actin positive cells as pericytes was confirmed by further analysis using antibodies specific for EC (EN-4) and an antibody against activated pericytes (HMWMAA). These results led the authors to suggest that pericytes or SMC may somehow contribute to the neovascular process. Examination of the localization of TGF-β in the cells of Kaposi's sarcomas (KS) revealed the precursor in both SMC and pericytes as well as in the spindle-shaped KS cells [144]. An autocrine as well as paracrine function was suggested for TGF-β in this pathology.

7. Summary

Though the functions of pericytes in vivo have yet to be clearly defined, their location on the abluminal surface of the microvascular EC — the tissue-blood interface — places them in a pivotal position. Elevated awareness of pericytes and increasing information obtained via investigation of them in vitro will undoubtedly lead to a better understanding of their role(s) in microvascular function under both normal and pathologic conditions.

Acknowledgements

References

[1] Eberth CJ. In: Handbuch der Lehre von den Geweben des Menschen und der Theire. Leipzig, 1871.

[2] Rouget C. Memoire sur le developpement, la structure et les proprietes physiologiques des capillaries sanguins et lymphatiques. Arch Physiol Normale Pathol 1873;5:603–61.

[3] Rouget C. Sur la contractilité des capillaires sanguins. CR Acad Sci 1879;88:916–8.

[4] Zimmerman K. Der Peinere Bau der Blutcapillaren. A Anat Entwicklungsgesch 1923;68:29–109.

[5] Epling GP. Electron microscopic observations of perictyes of small blood vessels in the lungs and hearts of normal cattle and swine. Anat Rec 1966;155:513–30.

[6] Sims DE. The pericyte — a review. Tissue Cell 1986;18:153–74.

[7] Weibel ER. On pericytes, particularly their existence on lung capillaries. Microvasc Res 1974;8:218–35.

[8] Mandarino LJ, Sundarraj N, Finlayson J, Hassell JR. Regulation of fibronectin and laminin synthesis by retinal capillary endothelial cells and pericytes in vitro. Exp Eye Res 1993;57:609–21.

[9] Tilton RG, Kilo C, Williamson JR. Pericyte–endothelial relation-

[10] Williamson JR, Tilton RG, Kilo C, Y S. Immunofluorescent imaging of capillaries and pericytes in human skeletal muscle and retina. Microvasc Res 1980;20:233–41.

[11] Mazanet R, Franzini-Armstrong C. Scanning electron microscopy of pericytes in rat red muscle. Microvasc Res 1982;23:361–9.

[12] Rhodin J. Ultrastructure of mammalian venous capillaries, venules, and small collecting veins. J Ultrastruct Res 1968;25:425–500.

[13] Meyrick B, Reid L. Ultrastuctural features of the distended pulmonary arteries of the normal rat. Anat Rec 1979;193:71–98.

[14] Simionescu M, Simionescu N, Palade GE. Permeability of muscle capillaries to small heme peptides. Evidence for the existence of patent transendothelial channels. J Cell Biol 1975;64:586–607.

[15] Le Beaux YJ, Willemot J. Actin- and myosin-like filaments in rat brain pericytes. Anat Rec 1978;190:811–199.

[16] Wallow IH, Burnside B. Actin filaments in retinal pericytes and endothelial cells. Invest Ophthalmol Vis Sci 1980;19:1433–41.

[17] Courtoy P, Boyles J. Fibronectin in the microvasculature: localization in the pericyte–endothelial interstitium. J Ultrastruct Res 1983;83:691–6.

[18] Herman IM, D'Amore PA. Microvascular pericytes contain muscle and nonmuscle actins. J Cell Biol 1985;101:43–52.

[19] DeNofrio D, Hook TC, Herman I. Functional sorting of actin isoforms in microvascular pericytes. J Cell Biol 1989;109:191–202.

[20] Skalli O, Pelte M-F, Feclet M-C, et al. Alpha-smooth muscle cell actin, a differentiation market of smooth muscle cells, is present in microfilamentous bundle of pericytes. J Histochem Cytochem 1989;37:315–21.

[21] Nehls V, Drenckhahn D. Heterogeneity of microvascular pericytes for smooth muscle type alpha-actin. J Cell Biol 1991;113:147–54.

[22] Herman IM. Controlling the expression of smooth muscle contractile protein isoforms: a role for the extracellular matrix? Am J Respir Cell Mol Biol 1993;9:3–4.

[23] Herman IM, Divaris N, Healy AM, Hoock TM. Regulation of pericyte growth and contractile phenotype by endothelial matrix and its associated growth factors. J Cell Biol 1991;115:443a.

[24] Ehler E, Karlhuber G, Bauer HC, Draeger A. Heterogeneity of smooth muscle-associated proteins in mammalian brain microvasculature. Cell Tiss Res 1995;279:393–403.

[25] Boado RJ, Pardridge WM. Differential expression of alpha-actin mRNA and immunoreactive protein in brain microvascular pericytes and smooth muscle cells. J Neurosci Res 1994;39:430–5.

[26] Joyce NC, Haire MF, Palade GE. Contractile proteins in pericytes. I. Immunoperoxidase localization of tropomyosin. J Cell Biol 1985;100:1379–86.

[27] Joyce NC, Haire MF, Palade GE. Contractile proteins in pericytes. II. Immunocytochemical evidence for the presence of two isomyosins in graded concentrations. J Cell Biol 1985;100:1387–95.

[28] Joyce NC, DeCamilli P, Boyles J. Pericytes, like vascular smooth muscle cells, are immunocytochemically positive for cyclic GMP-dependent protein kinase. Microvasc Res 1984;28:206–19.

[29] Sims DE, Miller FN, Donald A, Horne MM, Edwards MJ. Interleukin-2 alters the positions of capillary and venule pericytes in rat cremaster muscle. J Submicrosc Cytol Pathol 1994;26:507–13.

[30] Kelley C, D'Amore P, Hechtman HB, Shepro D. Microvascular pericyte contractility in vitro: a comparison with other cells of the vascular wall. J Cell Biol 1987;104:483–90.

[31] Kelley C, D'Amore P, Hechtman HB, Shepro D. Vasoactive hormones and cAMP affect pericyte contraction and stress fibers in vitro. J Muscle Res Cell Motil 1987;9:184–94.

[32] Das A, Frank RN, Weber ML, et al. ATP causes retinal pericytes to contract in vitro. Exp Eye Res 1988;46:349–62.

[33] Masaki T. Possible role of endothelin in endothelial regulation of vascular tone. Annu Rev Pharmacol Toxicol 1995;35:235–55.

[34] Takahashi K, Brooks RA, Kanse SM, et al. Production of endothelin 1 by cultured bovine retinal endothelial cells and presence of

endothelin receptors on associated pericytes. Diabetes 1989;38:1200–2.

[35] Busse R, Fleming I, Schini VB. Nitric oxide formation in the vascular wall: regulation and functional implications. Curr Topics Microbiol Immunol 1995;196:7–18.

[36] Haefliger IO, Zschauer A, Anderson DR. Relaxation of retinal pericyte contractile tone through the nitric oxide–cyclic guanosine monophosphate pathway. Invest Ophthalmol Vis Sci 1994;35:991–7.

[37] Rhodin JAG, Fujita H. Capillary growth in mesentery of normal young rats. Intravital video and electron microscope analyses. J Submicrosc Cytol Pathol 1989;21:1–34.

[38] Richardson RL, Hausman GJ, Campion DR. Response of pericytes to thermal lesion in the inguinal fat pad of 10-day-old rats. Acta Anat 1982;114:41–57.

[39] Hausman GJ, Campion DR, Martin RJ. Search of the adipocyte precursor cell and factors that promote its differentiation. J Lipid Res 1980;21:657–70.

[40] Brighton CT, Hunt RM. Ultrastructure of electrically-induced osteogenesis in the rabbit medullary canal. J Orthoped Res 1986;4:27–36.

[41] Brighton CT, Lorich DG, Kupcha R, et al. The pericyte as a possible osteoblast progenitor cell. Clin Orthoped 1992;275:287–99.

[42] Diaz-Flores L, Gutierrez R, Lopez-Alonso A, Gonzalez R, Varela H. Pericytes as a supplementary source of osteoblasts in periosteal osteogenesis. Clin Orthopaed Rel Res 1992;275:280–6.

[43] Maxwell DS, Kruger L. Small blood vessels and the origin on phagocytes in the rat cerebral cortex following heavy particle irradiation. Exp Neurol 1965;12:33–54.

[44] Mori S, Leblond CP. Identification of microglia in light and electron microscopy. J Comp Neurol 1969;135:57–80.

[45] Vaughn JE, Pease DC. Electron microscopic studies of Wallerian degeneration in rat optic nerves. II. Astrocytes, oligodendrocytes and adventitial cells. J Comp Neurol 1970;140:207–26.

[46] Torvik A, Skjorten F. Electron microscopic observations on nerve cell regeneration and degeneration after axon lesions. II. Changes in glial cells. Acta Neuropathol 1971;17:265–82.

[47] Majno G, Palade GE, Schoefl GI. Studies on inflammation. II. The site of action of histamine and serotonin along the vascular tree: a topographic study. J Biophys Biochem Cytol 1961;11:607–26.

[48] Kristensson K, Olsson Y. Accumulation of protein tracers in pericytes of the central nervous system following systemic injection in immature mice. Acta Neurol Scand 1973;49:189–94.

[49] Mato M, Ookawara S, Kurihara K. Uptake of exogenous substances and marked infoldings of the fluorescent granular pericyte in cerebral fine vessels. Am J Anat 1980;157:329–32.

[50] Markov DV, Dimova RN. Ultrastructural alterations of rat brain microglial cells and pericytes after chronic lead poisoning. Acta Neuropathol 1974;28:25–35.

[51] Schlondorff D. The glomerular mesangial cell: an expanding role for a specialized pericyte. FASEB J 1987;1:272–81.

[52] Farquhar MG, Palade GE. Functional evidence for the existence of a third cell type in the renal glomerulus. J Cell Biol 1962;13:55–87.

[53] Crocker DJ, Murad TM, Greer JC. Role of the pericyte in wound healing. An ultrastructural study. Exp Mol Pathol 1970;13:51–65.

[54] Coffin JD, Poole TJ. Embryonic vascular development: immuno-histochemical identification of the origin and subsequent morphogenesis of the major vessel primordia in quail embryo. Development 1988;102:1–14.

[55] Pardanaud L, Yassine F, Dieterlen-Lièvre F. Relationship between vasculogenesis, angiogenesis and haemopoiesis during avian ontogeny. Development 1989;105:473–85.

[56] Doetschman TA, Gossler A, Kemler R. Blastocyst-derived embryonic stem cells as a model for embryogenesis. In: Future Aspects in Human In Vitro Fertilization. Berlin: Springer-Verlag, 1987:187–95.

[57] Noden DM. Embryonic origins and assembly of blood vessels. Am Rev Respir Dis 1989;140:1097–103.

[58] Nakamura H. Electron microscopic study of the prenatal development of the thoracic aorta in the rat. Am J Anat 1988;181:406–18.

[59] Ekblom P, Sariola H, Karkinen-Jaaskelainen M, Saxen L. The origin of the glomerular endothelium. Cell Differ 1982;11:35–9.

[60] Westermark B, Siegbahn A, Heldin C-H, Claesson-Welsh L. B-type receptor for platelet-derived growth factor mediates a chemotactic response by means of ligand-induced activation of the receptor protein-tyrosine kinase. Proc Natl Acad Sci USA 1990;87:128–32.

[61] Montesano R, Vassali JD, Baird A, Guillemin R, Orci L. Basic fibroblast growth factor induces angiogenesis in vitro. Proc Natl Acad Sci USA 1986;83:7297–301.

[62] Williams LT. Signal transduction by the platelet-derived growth factor receptor. Science 1989;243:1564–70.

[63] Holmgren L, Glaser A, Pfeifer-Ohlsson S, Ohlsson R. Angiogenesis during human extra-embryonic development involves the spatiotemporal control of PDGF ligand and receptor gene expression. Development 1991;113:749–54.

[64] Vlodavsky I, Folkman J, Sullivan R, et al. Endothelial cell-derived basic fibroblast growth factor: synthesis and deposition into subendothelial extracellular matrix. Proc Natl Acad Sci USA 1987;84:2292–6.

[65] Healy MA, Herman IM. Density-dependent accumulation of basic fibroblast growth factor in the subendothelial matrix. Eur J Cell Biol 1992;59:56–67.

[66] Muthukrishnan L, Warder E, McNeil PL. Basic fibroblast growth factor is efficiently released from a cytosolic storage site through plasma membrane disruptions of endothelial cells. J Cell Physiol 1991;148:1–16.

[67] Bashkin P, Doctrow S, Klagsbrun M, et al. Basic fibroblast growth factor binds to subendothelial extracellular matrix and is released by heparitinase and heparin-like molecules. Biochemistry 1989;28:1737–43.

[68] D'Amore PA, Smith SR. Growth factor effects on cells of the vascular wall: a survey. Growth Factors 1993;8:61–75.

[69] Sato Y, Hamanaka R, Ono J, et al. The stimulatory effect of PDGF on vascular smooth muscle cell migration is mediated by the induction of endogenous basic FGF. Biochem Biophys Res Commun 1991;174:1260–6.

[70] Higashiyama S, Abraham JA, Miller J, Fiddes JC, Klagsbrun M. A heparin-binding growth factor secreted by macrophage-like cells that is related to EGF. Science 1991;251:936–9.

[71] Higashiyama S, Abraham JA, Klagsbrun M. Heparin-binding EGF-like growth factor stimulation of smooth muscle cell migration: dependence of interactions with cell surface heparan sulfate. J Cell Biol 1993;122:933–40.

[72] Yoshizumi M, Kourembanas S, Temizer DH, et al. Tumor necrosis factor increases transcription of the heparin-binding epidermal growth factor-like gene in vascular endothelial cells. J Biol Chem 1992;267:9467–9.

[73] Bradham DM, Igarashi A, Potter RL, Grotendorst GR. Connective tissue growth factor: a cysteine-rich mitogen secreted by human vascular endothelial cells is related to the SRC-induced immediate early gene product CEF-10. J Cell Biol 1991;114:1285–94.

[74] Carlson EC, Bjork NJ. SEM and TEM analyses of isolated human retinal microvessel basement membranes in diabetic retinopathy. Anat Rec 1990;226:285–306.

[75] Toma R. Über die Intima der Arterien. Virchows Arch 1921;230:1–45.

[76] Schwartz SM, Liaw L. Growth control and morphogenesis in the development and pathology of arteries. J Cardiovasc Pharmacol 1993;21:531–9.

[77] Antonelli-Orlidge A, Saunders KB, Smith SR, D'Amore PA. An activated form of TGF-β is produced by co-cultures of endothelial cells and pericytes. Proc Natl Acad Sci USA 1989;86:4544–8.

[78] Desmouliere A, Genioz A, Gabbiani F, Gabbiani G. Transforming

growth factor-β1 induces α-smooth muscle cell actin expression in granulation tissue myofibroblasts and in quiescent and growing cultured fibroblasts. J Cell Biol 1993;122:103–11.

[79] Verbeek MM, Otte-Höller I, Wesseling P, Ruiter DJ, de Waal RMW. Induction of α-smooth muscle actin expression in cultured human brain pericytes by transforming growth factor-β1. Am J Pathol 1994;144:372–82.

[80] Dickson MC, Martin JS, Cousins FM, et al. Defective haematopoiesis and vasculogenesis in transforming growth factor-β1 knock-out mice. Development 1995;121:1845–54.

[81] D'Amore PA. Mechanisms of endothelial growth control. Am J Respir Cell Mol Biol 1992;6:1–8.

[82] Dodge A, Lu X, D'Amore P. Density-dependent endothelial cell production of an inhibitor of smooth muscle cell growth. J Cell Biochem 1993;53:21–31.

[83] Larson DM, Carson MP, Haudenschild CC. Junctional transfer of small molecules in cultured bovine brain microvascular endothelial cells and pericytes. Microvasc Res 1987;34:184–99.

[84] Sweet E, Abraham EH, D'Amore PA. Functional evidence of gap junctions between capillary endothelial cells and pericytes in vitro. Invest Ophthalmol Vis Sci 1988;29:109a.

[85] Guthrie SC, Gilula NB. Gap junctional communication and development. TINS 1989;12:12–18.

[86] Reaume AG, de Sousa PA, Kulkarni S, et al. Cardiac malformation in neonatal mice lacking connexin 43. Science 1995;267:1831–1834.

[87] Segal SS, Bény J-L. Intracellular recording and dye transfer in arterioles during blood flow control. Am J Physiol 1992;263:H1–H7.

[88] Little TL, Xia J, Duling BR. Dye tracers define differential endothelial and smooth muscle coupling patterns within the arteriolar wall. Circ Res 1995;76:498–504.

[89] Elfgang C, Eckert R, Lichtenberg-Frate H, et al. Specific permeability and selective formation of gap junction channels in connexin-transfected HeLa cells. J Cell Biol 1995;129:805–17.

[90] Bruzzone R, Haefliger J-A, Gimlich RL, Paul DL. Connexin 40, a component of gap junctions in vascular endothelium, is restricted in its ability to interact with other connexins. Mol Biol 1993;4:7–20.

[91] Traub O, Eckert R, Lichtenberg-Frate H, et al. Immunochemical and electrophysiological characterization of murine connexin 40 and -43 in mouse tissues and transfected human cells. Eur J Cell Biol 1994;64:101–12.

[92] Little TL, Beyer EC, Duling BR. Connexin 43 and connexin 40 gap junctional proteins are present in arteriolar smooth muscle and endothelium in vivo. Am J Physiol 1995;268:H729–H39.

[93] Reed KE, Westphale EM, Larson DM, et al. Molecular cloning and functional expression of human connexin 37, an endothelial gap junction protein. J Clin Invest 1993;91:997–1004.

[94] Lash JA, Critser ES, Pressler ML. Cloning of a gap junctional protein from vascular smooth muscle and expression in two-cell mouse embryos. J Biol Chem 1990;265:13113–7.

[95] Moore LK, Beyer EC, Burt JM. Characterization of gap junction channels in A7r5 vascular smooth muscle cells. Am J Physiol 1991;260:C975–C81.

[96] White T, Paul D, Goodenough D, Bruzzone R. Functional analysis of selective interactions among rodent connexins. Mol Biol Cell 1995;6:459–70.

[97] Jongen WMF, Fitzgerald DJ, Asamoto M, et al. Regulation of connexin 43-mediated gap junctional intercellular communication by Ca^{2+} in mouse epidermal cells is controlled by E-cadherin. J Cell Biol 1991;114:545–55.

[98] Musil LS, Cunningham BA, Edelman GM, Goodenough DA. Differential phosphorylation of the gap junction protein connexin 43 in junctional communication-competent and -deficient cell lines. J Cell Biol 1990;111:2077–88.

[99] Suzuki S, Sano K, Tanikara H. Diversity of the cadherin family: evidence for eight new cadherins in nervous tissue. Cell Reg 1991;2:261–70.

[100] Liaw CW, Cannon C, Power MD, Kiboneka PK, Rubin LL. Identification and cloning of two species of caherins in bovine endothelial cells. EMBO J 1990;9:2701–8.

[101] Rubin L, Hall D, Porter S, et al. A cell culture model of the blood–brain barrier. J Cell Biol 1991;115:1725–35.

[102] Solomon D, Ayalon D, Patel-King R, Hynes RO, Geiger B. Extrajunctional distribution of N-cadherin in cultured human endothelial cells. J Cell Sci 1992;192:7–17.

[103] Gilbertson-Beadling SK, Fisher C. A potential role for N-cadherin in mediating endothelial cell–smooth muscle cell interactions in the rat vasculature. Lab Invest 1993;69:203–9.

[104] Verbeek MM, Westphal JR, Ruiter DJ, de Waal RM. T lymphocyte adhesion to human brain pericytes is mediated via very late antigen-4/vascular cell adhesion molecule-1 interactions. J Immunol 1995;154:5876–84.

[105] Newman PJ, Berndt MC, Gorsk J, White GC II, Lyman S. Molecular cloning of PECAM-1 [CD31]: a novel member of the immunoglobin gene superfamily expressed on human platelet and endothelial cell intercellular junctions. Science 1990;247:1219–22.

[106] Tedder TF, Steeber DA, Chen A, Engel P. The selectins: vascular adhesion molecules. FASEB J 1995;9:866–73.

[107] Vora AJ, Perry ME, Hobbs C, Dumonde DC, Brown KA. Selective binding of peripheral blood lymphocytes to the walls of cerebral vessels in frozen sections of human brain. J Immunol Meth 1995;180:165–80.

[108] Hynes RO. Integrins: versatility, modulation, and signaling in cell adhesion. Cell 1992;60:11–25.

[109] Sastry S, Horwitz AF. Integrin cytoplasmic domains: mediators of cytoskeletal linkages and extra- and intracellular initiated transmembrane signaling. Curr Opin Cell Biol 1993;5:819–31.

[110] D'Amore PA. Culture and study of pericytes. In: Cell Culture Techniques in Heart and Vessel Research. Berlin: Springer-Verlag, 1990:299–314.

[111] Barrett TB, Gajdusek CM, Schwartz SM, McDougall JK, Benditt EP. Expression of the sis gene by endothelial cells in culture and in vivo. Proc Natl Acad Sci USA 1984;81:6772–4.

[112] Collins T, Pober JS, Gimbrone MA, et al. Cultured human endothelial cells express platelet-derived growth factor A chain. Am J Pathol 1987;126:7–12.

[113] Collins T, Ginsburg D, Boss JM, Orkin SH, Pober JS. Cultured human endothelial cells express platelet-derived growth factor B chain: cDNA cloning and structural analysis. Nature 1985;316:748–50.

[114] Folkman J, Haudenschild CC, Zetter BR. Long-term culture of capillary endothelial cells. Proc Natl Acad Sci USA 1979;76:5217–21.

[115] Heimark RL, Twardzik DR, Schwartz SM. Inhibition of endothelial cell regeneration by type-beta transforming growth factor from platelets. Science 1986;233:1078–80.

[116] Cohen MP, Frank RN, Khalifa AA. Collagen production by cultured retinal pericytes. Invest Ophthalmol Vis Sci 1980;19:90–94.

[117] Stramm LE, Li W, Aguirre GD, Rockey JH. Glycosaminoglycan synthesis and secretion by bovine retinal capillary pericytes in culture. Exp Eye Res 1987;44:17–28.

[118] Orlidge A, D'Amore PA. Inhibition of capillary endothelial cell growth by pericytes and smooth muscle cells. J Cell Biol 1987;105:1455–62.

[119] Sato Y, Rifkin DB. Inhibition of endothelial cell movement by pericytes and smooth muscle cells: activation of a latent transforming growth factor-beta 1-like molecule by plasmin during co-culture. J Cell Biol 1989;109:309–15.

[120] Dennis PA, Rifkin DB. Cellular activation of transforming growth

factor β requires binding to the cation-independent mannose 6-phosphate/insulin-like growth factor type II receptor. Proc Natl Acad Sci USA 1991;88:580–4.

[121] Fillinger MF, O'Connor SE, Wagner RJ, Cronenwett JL. The effect of endothelial cell co-culture on smooth muscle cell proliferation. J Vasc Surg 1993;17:1058–68.

[122] Folkman J, Haudenschild C. Angiogenesis in vitro. Nature 1980;288:551–6.

[123] Montesano R, Orci L. Tumor-promoting phorbol esters induce angiogenesis in vitro. Cell 1985;42:469–77.

[124] Nicosia RF, Ottinetti A. Modulation of microvascular growth and morphogenesis by reconstituted basement membrane gel in three dimensional cultures of rat aorta: a comparative study of angiogenesis in matrigel, collagen, fibrin, and plasma clot. In Vitro Cell Dev Biol 1990;26:119–28.

[125] Nehls V, Schuchardt E, Drenckhahn D. The effect of fibroblasts, vascular smooth muscle cells, and pericytes on sprout formation of endothelial cells in a fibrin gel angiogenesis system. Microvasc Res 1994;48:349–63.

[126] Jaye M, McConathy E, Drohan W, et al. Modulation of the sis gene transcript during endothelial cell differentiation in vitro. Science 1985;228:882–5.

[127] Yamagishi S-I, Kobayashi K-I, Yamamoto H. Vascular pericytes not only regulate growth, but also preserve prostacyclin-producing ability and protect against lipid peroxide-induced injury of co-cultured endothelial cells. Biochem Biophys Res Commun 1993;190:413–25.

[128] Davies PF, Loesen S-P, Clapham DE, Morrel EM, Schoen FJ. Endothelial communication. Hypertension 1988;11:563–72.

[129] Davies PF, Truskey GA, Warren HB, O'Connor SE, Eisenhaure BH. Metabolic cooperation between vascular endothelial and smooth muscle cells in co-culture: changes in low density lipoprotein metabolism. J Cell Biol 1985;101:871–9.

[130] Sundberg C, Ljungström M, Lindmark G, Gerdin B, Rubin K. Microvascular pericytes express platelet-derived growth factor-β receptors in human healing wounds and colorectal adenocarcinoma. Am J Pathol 1993;143:1377–88.

[131] Yamagishi S, Hsu C-C, Kobayashi K, Yamamoto H. Endothelin 1 mediates endothelial cell-dependent proliferation of vascular pericytes. Biochem Biophys Res Commun 1993;191:840–6.

[132] Newcomb P, Herman IM. Pericyte growth and contractile phenotype: modulation by endothelial-synthesized matrix and comparison with aortic smooth muscle cells. J Cell Phys 1993;155:385–93.

[133] Davies P, Smith BT, Maddalo FB, Langleben D, Tobias D, Fujiwara K, Reid L. Characteristics of lung pericytes in culture including their growth inhibition by endothelial substrate. Microvasc Res 1987;33:300–14.

[134] Herman IM. Microvascular pericytes in development and disease. In: The Blood–Brain Barrier. New York: Raven Press, 1993:127–35.

[135] Sims DE. Recent advances in pericyte biology — implications for health and disease. Can J Cardiol 1991;7:431–43.

[136] Herman IM, Jacobson S. In situ analysis of microvascular pericytes in hypertensive rat brains. Tissue Cell 1988;20:1–12.

[137] Tilton RG, Faller AM, Hoffman PL, Kilo C, Williamson JR. Acellular capillaries and increased pericyte degeneration in the diabetic extremity. Front Diabetes 1987;8:186–9.

[138] Giannini C, Dyck PJ. Basement membrane reduplication and pericyte degeneration precede development of diabetic polyneuropathy and are associated with its severity. Ann Neurol 1995;37:498–505.

[139] Robison WG, McCaleb ML, Feld LG, et al. Degenerated intramural pericytes ("ghost cells") in the retinal capillaries of diabetic rats. Curr Eye Res 1991;10:339–50.

[140] Robison WG, Nagata M, Laver N, Hohman TC, Kinoshita JH. Diabetic-like retinopathy in rats prevented with an aldose reductase inhibitor. Invest Ophthal Vis Sci 1989;30:2285–92.

[141] Robison WG, Tisslis TN, Laver N, Kinoshita JH. Diabetes-related histopathologies of the rat retina prevented with an aldose reductase inhibitor. Exp Eye Res 1990;50:335–66.

[142] Kuwabara T, Cogan DG. Retinal vascular patterns. VI. Mural cells of the retinal capillaries. Arch Ophthalmol 1963;69:492–502.

[143] Wesseling P, Schlingemann RO, Rietveld FJ, et al. Early and extensive contribution of pericytes/vascular smooth muscle cells to microvascular proliferation in glioblastoma multiforme: an immuno-light and immuno-electron microscopic study. J Neuropathol Exp Neurol 1995;54:304–10.

[144] Williams AO, Ward JM, Jackson MA, Flanders KC. Immunohistochemical localization of transforming growth factor-beta1 in Kaposi's sarcoma. Human Pathol 1995;26:469–73.