Chapter 13
Pericytes in Atherosclerosis

Volha Summerhill and Alexander Orekhov

Abstract Pericytes are pluripotent cells found in the vascular wall of both capillaries and large blood vessels. Pericytes are highly heterogeneous cells in terms of phenotype, tissue distribution, origin and functions, and they play an important role in the regulation of vascular morphogenesis and function. Pericytes were shown to be involved in tissue development and homeostasis, as well as in pathological processes, including atherosclerosis. Both microvascular and macrovascular pericytes form the cellular network of the arterial wall and are actively involved in lipid accumulation, growth, and neovascularization of the atherosclerotic plaque, local inflammation and thrombosis. According to current understanding, pericytes originate from the multipotent stem cells capable of mesenchymal differentiation to oligopotent lineages, such as osteoclasts, chondrocytes, and adipocytes, and also serve as mesenchymal local progenitors in tissues. Pericyte multilineage potential is fundamental for vascular pathology, including atherosclerotic lesion formation. Pericytes express various surface proteins that can be used for their identification in aid of diagnosis and therapeutic strategies for atherosclerosis and other vascular pathologies.

Keywords Pericyte · Atherosclerosis · Cardiovascular diseases · Mesenchymal stem cells · Endothelial cells · Vascular smooth muscle cells · Endothelial dysfunction · Angiogenesis · Differentiation · Blood vessels · Foam cells · Low-density lipids

V. Summerhill · A. Orekhov (✉)
Laboratory of Angiopathology, Institute of General Pathology and Pathophysiology, Moscow, Russia

Institute for Atherosclerosis Research Moscow, Skolkovo Innovative Center, Moscow, Russia

© Springer Nature Switzerland AG 2019

A. Birbrair (ed.), *Pericyte Biology in Disease*, Advances in Experimental Medicine and Biology 1147, https://doi.org/10.1007/978-3-030-16908-4_13

Introduction

Coronary artery disease (CAD) as a consequence of atherosclerosis is a leading cause of morbidity and mortality worldwide (Mozaffarian et al. 2015). Atherosclerotic lesion development occurs in the arterial subendothelial intima, especially in the regions of perturbed blood flow, such as vessel bifurcations. The pathological process is characterized by an interplay between the endothelial dysfunction and subendothelial lipid accumulation, resulting in chronic inflammation in the arterial wall (Tabas et al. 2015). In that regard, cell types that can participate in these processes, and therefore provide the potential targets for therapeutic intervention, attract special attention. Among the vascular wall cell types, pericytes are the most enigmatic due to their phenotypic variety and multifunctionality. Pericytes are able to differentiate into several cell lineages, and, therefore, contribute to the formation, maturation, and homeostasis of the entire human vascular bed (Montiel-Eulefi et al. 2012). Moreover, pericytes vary functionally and morphologically between different organs and tissues, adding to the complexity of the vascular wall composition. Thus, for example, central nervous system (CNS) pericytes are functionally distinct from the peripheral blood vessels pericytes (Wang et al. 1992; Balabanov and Dore-Duffy 1998). Pericyte content also varies considerably between different tissues and organs. For instance, these cells are more numerous in the walls of larger blood vessels that must maintain higher blood pressure. According to Sims (2000), the endothelial cell-to-pericyte ratio is the highest (1:1) in the CNS and retina. Significantly lower proportions were reported for lung and skin tissues (1:10) and skeletal muscles (1:100). However, these cells have proven difficult to identify and quantify precisely due to their high heterogeneity and the lack of a single specific marker. Because of these challenges, the role of pericytes in health and disease remains to be studied in detail (Krueger and Bechmann 2010).

In this chapter, we will discuss the morphological and functional significance of pericytes in vascular homeostasis and their involvement in various aspects of atherosclerosis pathogenesis, such as endothelial dysfunction, angiogenesis (neovascularization of plaques), local inflammation, calcification, and thrombosis. We will also revise current and evolving concepts on the pericyte ontogeny.

Functional and Morphological Features of Pericytes

In the blood vessel wall, pericytes are located in the proximity of the endothelial cells, in the basal membrane. They are characterized by a prominent round nucleus and a relatively small content of cytoplasm. Pericytes form contacts with endothelial cells of the capillary wall, as well as with other cells and with each other, by means of long cytoplasmic processes. These contacts play important role in maintaining the endothelial structure and function (Mazanet and Franzini-Armstrong 1982; Rucker et al. 2000). Recent experiments revealed the regulatory and signalling activities of pericytes towards the endothelium. For instance, pericytes were

shown to inhibit endothelial cell division via activation of TGF-β (Betsholtz et al. 2005). Gap junction contacts formed by pericytes permit interchange of ions and small molecules, including N-cadherin, fibronectin, connexin and various integrins (Bergers and Song 2005; Armulik et al. 2005; Dore-Duffy and Cleary 2011). These endothelium–pericyte interactions are functionally associated with active regulation of blood flow throughout the body, hence, blood pressure that can be particularly important in vascular adaptation to hypotoxic injury. Moreover, in capillaries, pericytes participate in controlling the blood flow by their contractile ability, conveyed by the expression of varying amounts of essential contractile proteins, such as actin, myosin, smooth muscle actin alpha (SMA-α), tropomyosin and desmin (Bandopadhyay et al. 2001). Pericyte participation in regulating the vessel diameter was observed in retinal and medullary microvessels in response to depolarization and neuronal activity (Wu et al. 2003; Peppiatt et al. 2006). Moreover, several vasoactive molecules that regulate pericyte contraction and relaxation have been identified, including cholinergic α-2 and adrenergic β-2 receptors, angiotensin II and endothelin-1 (Bergers and Song 2005). Noteworthy, pericyte contractility can be important for regulation of capillary blood flow in pathological conditions, such as ischaemia (Yemisci et al. 2009; Hall et al. 2014). However, in the aorta and other large blood vessels, the contractile ability of pericytes is not that important, as the vascular tone is maintained by other mechanisms (Orekhov et al. 2016a). Physiological functions of perivascular pericytes that are known to date are listed in Table 13.1.

The diversity of pericyte biological functions is reflected by the numerous abnormalities of these cells associated with pathological conditions. Vascular disorders, including atherosclerosis, are accompanied by such alterations as abnormal count and size of endothelial cell–pericyte interfaces, excess or deficiency in pericyte coverage, and changes in pericyte contractility. These changes, in turn, lead to altered vessel sprouting, remodelling, maturation and stabilization (Gerhardt and Betsholtz 2003; Hughes et al. 2006).

Table 13.1 Functions of perivascular pericytes

Function	References
Angiogenesis and vessel stabilization	Betsholtz et al. (2005), Raza et al. (2010)
Capillary blood flow regulation	Cuevas et al. (1984), Pallone et al. (1998, 2003), Stefanska (2013)
Vascular morphogenesis, maturation and remodelling	Leveen et al. (1994), Hellstrom et al. (2001), Chen et al. (2007), Herman (2010), Warmke et al. (2016)
Vascular permeability	Glentis et al. (2014)
Maintenance functional integrity of the blood–brain barrier	Dohgu et al. (2005), Peppiatt et al. (2006), Koh et al. (2008), Krueger and Bechmann (2010), Al Ahmad et al. (2011)
Blood coagulation (thrombosis)	Bouchard et al. (1997), Kim et al. (2006), Dulmovits and Herman (2012)
Lymphocyte activation	Thomas (1999), Bose et al. (2013)
Phagocytosis	Guillemin and Brew (2004)

Methods of Pericyte Identification

Being pluripotent heterogeneous cells, pericytes are difficult to identify in organs and tissues. Only a few markers of pericytes have been established, and none of them is universal, hence specific for all pericyte subtypes and under any circumstances. The expression patterns of the pericyte antigens are tissue-specific and can be up- or downregulated in pathological conditions. Moreover, the expression of some of the markers depends on the developmental stage of a blood vessel. Cell culture conditions can also influence the expression of marker proteins in pericytes cultured in vitro.

Many of the proteins expressed by pericytes are expressed in other cell types as well. The most obvious example is smooth muscle actin (SMA-α), which is a typical protein in vascular smooth muscle cells (SMCs) (van Dijk et al. 2015). It was found that vascular pericytes are often positive for SMA-α, but SMA-α-negative pericytes can also be observed (Bandopadhyay et al. 2001). For instance, a population of SMA-α-positive intimal cells was found that expressed CD68, protein atypical for vascular SMCs, which is usually regarded as a macrophage marker (Andreeva et al. 1997b). Interestingly, the ratio of SMA-α+CD68+ (double positive) cells was increased in atherosclerotic lesions, as well as in primary cell culture exposed to atherogenic-modified low-density lipids (LDL) (Orekhov et al. 1998). Another example is platelet-derived growth factor receptor beta (PDGFR-β), which, apart from pericytes, is also present in fibroblasts, astrocytes, and some tumour cells (Lindahl et al. 1997). Furthermore, pericyte-like cells in the human aortic intima express S-100 and CD1, which are common for dendritic cells (Bobryshev and Lord 2000; Bobryshev et al. 2011).

Hence, immunocytochemical identification of pericytes should be performed using combinations of markers. The markers proposed for common pericyte identification include SMA-α, PDGFR-β, aminopeptidase A and N (CD13), neuron-glial 2 (NG2), and desmin (Armulik et al. 2011). Other potential pericyte markers are CD146, endoglin, non-muscle myosin, nestin, vimentin, O-sialoganglioside 3G5, and melanoma chondroitin sulphate proteoglycan 2A7 (Nazarova et al. 1995; Morikawa et al. 2002; Dondossola et al. 2013; Ivanova et al. 2015; Rossi et al. 2016; Chen et al. 2017).

Origin of Vascular Pericytes

Lineage-tracing studies showed that pericytes' ontogeny is rather complex. Pericytes are considered to be pluripotent stem cells that exhibit multilineage developmental features of mesenchymal stromal stem cell (MSC). MSCs are involved in the maintenance of high turnover tissues like liver, skin, skeletal muscles, adipose tissue, and dental pulp. Furthermore, MSC participates in neurogenesis, which probably confirms the neuroectodermal origin of CNS pericytes (Nakata et al. 2017). Similarly, during developmental stages, neuroectodermal cells can possibly differentiate into

vascular SMCs of embryonic cerebral vessels (Korn et al. 2002). To achieve the alignment in MSC identification across different studies, International Society for Cellular Therapy created a position statement formulating the MSC minimum regular criteria. First, MSCs are adherent to plastic when maintained in standard culture conditions. Second, MSCs can be characterized by specific surface antigens (positive for CD105, CD73, and CD90 and negative for CD45, CD34, CD14, CD11b, CD79α, CD19, or HLA-DR). Third, MSCs are able to differentiate into osteoblasts, adipocytes, and chondroblasts *in vitro* (Dominici et al. 2006).

It was shown that vascular pericytes may serve as a source for local mesenchymal progenitor cells in adults (Crisan et al. 2007). Numerous *in vivo* and *in vitro* experiments demonstrated pericyte ability for differentiation into several lineages, giving rise to osteoblasts, chondrocytes, adipocytes, SMCs, fibroblasts, and Leydig cells (Kirton et al. 2006; Birbrair et al. 2015). Pericytes can give rise to multiple mesodermal tissues in situ, in response to PDGFR-β (Bouacida et al. 2012).

Direct relationship between MSC and perivascular pericytes was established based on the common expression markers and differentiation abilities of these cell types. This supported the point of view on pericytes as potential progenitor cells with wide differentiation capacity continuously present in developed adult tissues (Chen et al. 2009). In this regard, it is important to understand whether MSC, multipotent adult progenitor cells (MAPC), muscle-derived stem cells (MDSC), and adipose tissue-derived stem cells share a common progenitor in multiple developed organs. Studies of these progenitor cell lineages were hindered by the fact that all of them have only been identified and studied in primary cultures of donor tissues.

Several independent studies described novel subsets of endothelial cells in embryonic tissues that contribute to blood vessel formation and develop into non-vascular cell lineages promoting post-natal growth and regeneration of tissues. For instance, vascular endothelium generating embryonic haematopoietic cells and pluripotent mesoangioblasts have been derived from the aorta and other blood vessels of the embryo (Oberlin et al. 2002; Zambidis et al. 2006; Chen et al. 2009; Zheng et al. 2007).

The relationship between pericytes and MSCs was studied in more details both in situ and in vitro (Crisan et al. 2008). To that end, a combination of markers expressed by perivascular pericytes in human foetal and adult organs was used. Pericytes were identified as cells positive for CD146, NG2, PDGFRβ, and ALP and negative for CD34, CD45, vWF, and CD144. It was found that human perivascular cells extracted from various tissues and brought to culture gave rise to adherent, multilineage precursor cells that exhibited apparent MSC features and could differentiate into osteogenic, chondrogenic, adipogenic, and myogenic lineages. These findings allowed making a provocative speculation that all MSCs are pericytes. The multilineage potential of pericytes may contribute to muscle regeneration, as well as fat accumulation (Birbrair et al. 2013; Kostallari et al. 2015).

However, at present, the understanding of these mural stem/progenitor populations, as well as their potential biologic function, is unclear. The hypothesis that any of these populations are associated with a stem cell niche remains to be supported experimentally (Kovacic and Boehm 2009; Murray et al. 2014).

Role of Pericytes in Atherosclerosis

Initially, pericytes were believed to be present only in capillaries, where they participate in contractility and angiogenesis. However, further studies revealed the presence of pericytes in the vascular wall of large arteries and veins, where they populate the subendothelial layer of the intima (Orekhov et al. 2014; Juchem et al. 2010). In these tissues, pericytes can be distinguished as stellar-shaped cells with multiple processes that form contacts with each other and with the adjacent endothelial cells, thereby creating a cellular network of the intima that may play important regulatory function. As mentioned above, in large blood vessels, the contractile function of pericytes is essentially lost, which is reflected by reduced or absent expression of SMA-α (Orekhov et al. 2016a). Moreover, in large blood vessels, pericytes are also present as part of vasa vasorum, the capillaries of the vascular wall (Andreeva et al. 1998; Campagnolo et al. 2010; Juchem et al. 2010).

Atherosclerotic lesion development is associated with profound qualitative and quantitative changes in the cellular composition of the affected site. Most of these changes occur in the innermost layer of the intima, involving the endothelium and subendothelial space. Microscopic studies revealed that in the affected area, the cellular network becomes disrupted, with reduction in cell-to-cell interaction ratio and the number of gap junctions. Such disruption is likely to be a consequence of lipid accumulation (Orekhov et al. 2016a). The extent of the intercellular interactions through the gap junctions can be estimated by the expression of cell surface protein connexin 43 (Cx43), which is an essential component of the intercellular contacts. In atherosclerotic lesions, the number of Cx43 plaques per cell was greater in lipid-free cells, compared to lipid-laden cells, and was decreasing towards the lumen, which was not the case in the grossly normal intima. These observations may indicate that intracellular lipid accumulation can have a causative role in pathological pericytes alterations and cellular network disruption (Orekhov et al. 2016a).

Early studies showed that atherosclerotic changes in the vascular wall are associated with the increase in a number of 3G5-positive pericyte-like cells in the proteoglycan-rich layer of the aortic intima (Nayak et al. 1988). Morphological analysis demonstrated that atherosclerotic lesions had a six-fold increase in stellate cell (likely pericytes) count in comparison to grossly normal areas, while the number of elongated cells (likely SMCs) and total cell count were increased by two-fold (Orekhov et al. 2014). A direct link was confirmed between the increased number of stellate cells and the content of cholesteryl esters and total lipids in the vascular wall (Orekhov et al. 2016b).

At advanced stages of atherosclerotic lesion development, neovascularization of the plaques becomes evident and plays an important role in the plaque growth. This process is likely to be mediated by T-cadherin signalling (Moreno et al. 2012). As stated by Collett and Canfield (2005), angiogenesis may influence atherosclerotic lesion formation in the following ways: (1) the expansion of new blood vessels in lipid-laden plaques modifies artery permeability, when thickening diminishes oxygen diffusion; (2) albumin and fibrinogen leaking into the artery wall contribute to

the plaque formation; (3) intimal microvessels are associated with haemorrhagic sites and ruptures on the plaques; (4) additionally, the presence of inflammatory cells, i.e. leukocytes, macrophages, and mast cells around neovessels of lipid-rich plaques, is indicative that these new vessels are immediately involved in the recruitment of inflammatory cells, therefore, lesion progression, calcification, and destabilization.

The finding that pericytes can differentiate into osteoblasts and chondrocytes is suggestive that they may be involved in the maladaptive ectopic calcification, occurring in atheromatous vessels, where they act as a source of osteoprogenitor cells in the lesions. This process is followed by matrix remodelling and increased recruitment of calcifying vascular cells (CVCs) (Collett and Canfield 2005). Interestingly, CVCs were identified in bovine aortic media as a phenotypic modulation of SMCs (Steitz et al. 2001). Similar to pericytes, CVCs also have the potential to differentiate along several mesenchymal cell lineages, but with a distinct differentiation pattern. Thus, CVCs may obtain osteogenic and chondrogenic phenotypes in the arterial intima, but, unlike pericytes, they do not reveal any potential for adipogenic differentiation. It can be hypothesized that CVCs may represent second-generation pluripotent cells that are transitional between the precursor and terminally differentiated mesenchymal cells (Tintut et al. 2003).

Ectopic calcification of blood vessels is a common feature of advanced atherosclerotic plaques. In association with pericytic chondrogenesis and osteogenesis, proteins, including osteopontin, osteoprotegerin, bone sialoprotein, matrix Gla protein, Axl, alkaline phosphatase, and bone morphogenetic protein-2 (BMP-2) and BMP-4, were detected in the aortic valve atherosclerotic lesions (Rajamannan et al. 2003).

Activation of adventitial cells was described in the following conditions: vein grafting, hypoxia, and hypertension. Under physical stress, adventitial cells may differentiate into myofibroblasts that migrate into the inner layers of the tunica adventitia, followed by the release of paracrine factors regulating vascular remodelling (Haurani and Pagano 2007). Corselli et al. (2012) demonstrated two independent perivascular MSC progenitors: pericytes in microvessels and adventitial cells around larger vessels persist, and hence adventitial cells are able to gain pericytes-like phenotype. Furthermore, Andreeva et al. (1997a) indicated that pericyte-like cells account for over 30% of total cell count of the intima. Also, they have pointed out that a network of 3G5 antigen-positive pericyte-like cells is present mainly in the subendothelial layer. These findings suggested that functions of macropericytes are determined by subendothelial localization and are similar to those of the capillary pericytes. In addition, 3G5-positive pericyte-like cells have been identified in the bovine aorta, in the human unaffected intima and in calcified atherosclerotic plaques (Bostrom et al. 1993). It should be noted that branching regions of larger vessels are most abundant in pericyte-like cells (Moore and Tabas 2011). In addition, Nicosia (2009) reported that pericytes contribute to organogenesis of the aorta in embryos.

It is well known that atherosclerotic lesion formation is triggered by the circulating modified LDL that provoke endothelial damage and accumulate in the arterial

wall. In turn, distorted morphology of the endothelial cells, frequently observed in the atherosclerosis-affected sites, compromises their function leading to the inadequate vasoconstriction, leukocyte infiltration, coagulation, increased permeability that facilitates LDL entry in the subendothelial space, and accelerated proliferation or apoptosis (Groschner et al. 2012; Favero et al. 2014). It is worth mentioning that the atherogenic effects of modified LDL are not limited to lipid accumulation. In fact, the numerous studies have demonstrated pro-apoptotic effects of oxidized LDL (oxLDL), i.e. the induction of oxidative stress that can disturb endothelial function and, in turn, promote atherosclerotic lesion formation (Mollace et al. 2015; Salvayre et al. 2002). In addition, the exposure to oxLDL caused apoptosis in cultured multipotent progenitor cells through their membrane damage (Li et al. 2014).

Since pericytes reside immediately below the endothelium and are essential for maintaining endothelial function (van Dijk et al. 2015), it is possible that they are engaged in the atherogenesis-induced endothelial alterations initiating atherosclerotic plaque formation (Fig. 13.1). It was established that lipid accumulation by the subendothelial cells of human aortic intima impairs cell-to-cell communications through the gap junctions in the proteoglycan-rich layer (Andreeva et al. 1991; Rekhter et al. 1993; Juchem et al. 2010). Furthermore, assuming that there is a connection between intracellular lipid content and the rate of cell-to-cell communication, Andreeva et al. (1995) using fluorescent dye injected into cells with and without visual lipid inclusions demonstrated that the proportion of lipid-free cells communicating via gap junctions was similar to that of cells cultured from grossly normal areas of intima. At the same time, the rate of intercellular communication of cells with lipid inclusions was two-fold lower, compared to that of lipid-free cells. In addition, the presence of lipid-rich cells resembling so-called foam cells was typical for cultures derived from atherosclerotic lesions (Andreeva et al. 1995).

It can be hypothesized that functional changes of the gap junctions are one of the causes of atherosclerosis-related breakdowns of cellular networks formed by the proteoglycan-rich layer intimal pericytes. However, the exact role of pericytes in the endothelial dysfunction associated with lipid accumulation is still vague. Endothelium is likely to be damaged from the luminal side by the disturbed blood flow, especially at branching sites, where blood flow is non-linear and undergoing shear stress leads to augmented inflammatory signalling and inflammatory cell recruitment (Pantakani and Asif 2015).

Based on the assumption that intracellular lipid accumulation caused by modified LDL is the crucial event in atherogenesis, it was suggested that aortic pericytes are capable of active lipid accumulation in the subendothelial layer of the arterial intima that changes their phenotype from dormant to proliferative (active) (Ivanova and Orekhov 2016). Moreover, when activated, pericytes may turn into aberrant differentiation to chondrogenic, osteogenic, macrophage, and myofibroblast lineages contributing to the pathogenesis of atherosclerosis and vascular calcification. Thus, it can be suggested that upon formation of the atherosclerotic lesions, the functional state of the pericytes in the proteoglycan-rich layer of the intima is changed and associated with variations in antigenic expression (Orekhov and Bobryshev 2015).

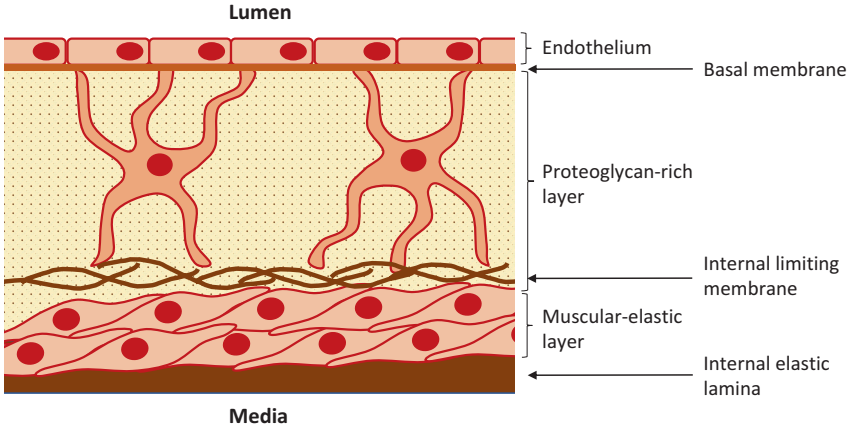

Fig. 13.1 Schema is presenting the organization of the arterial intima. The proteoglycan-rich layer containing a heterogeneous population of cells, including macrovascular pericytes, is located just below the endothelial monolayer. Intimal pericytes forming a network of cells linked via gap junctions. The muscular-elastic layer, formed by elongated contractile smooth muscular cells, is immediately below the proteoglycan-rich layer. Note: *World Journal of Cardiology*. 2015;7(10):583–593. Doi: https://doi.org/10.4330/wjc.v7.i10.583

Accordingly, the expression of 2A7 antigen is attributed to activated pericytes. That was proven by the presence of 2A7+ cells in atherosclerotic plaques using anti-2A7 antibody that adheres to activated pericytes, while no 2A7+ cells were identified in normal intima (Orekhov and Bobryshev 2015).

Moreover, acquiring the phagocytic phenotype, pericytes express CD68 macrophage-associated antigen, which is a scavenger receptor. These scavenger receptors of pericytes appear to promote uptake and accumulation of LDL, indicating their participation in active phagocytosis (Shashkin et al. 2005; Song et al. 2011; Cochain and Zernecke 2015). Such phagocytic ability of pericytes, i.e. engulfing of LDL particles, can lead to the development of foam cells and thickening of the arterial wall. In line with this suggestion, lipid-laden, stellate pericyte-like cells enriched by synthesizing organelles were found in atherosclerotic plaques (Orekhov et al. 1986; Andreeva et al. 1991, 1997b).

Further lipid accumulation via phagocytosis aggravates the situation in the atherosclerotic lesion leading to segregation of cells, breakdown of cell-to-cell contacts, and disruption of the cellular network. In addition, like fibroblasts, pericytes can rapidly proliferate accelerating thickening of the arterial wall and contributing to the extracellular matrix synthesis (Ivanova and Orekhov 2016).

The most pronounced changes associated with atherosclerosis occur in the proteoglycan-rich layer of the intima: lipid deposition and thickening determined by the accumulation of all types of collagen fibres with disrupted orientation, especially in the fibrous cap (Andreeva et al. 1997a, 1997b; Ivanova et al. 2015). Microscopic studies of atherosclerosis-affected and grossly normal aortic intima showed a number of distinct features: the presence of lipid droplets and vesicles

between the interstitial collagen fibres, thickened proteoglycan-rich layer with increased collagen content, and altered longitudinal alignment of collagen fibres was accompanied by the formation of thick capsules around subendothelial cells (Orekhov et al. 2016b). Notably, the total lipid content in the proteoglycan-rich layer was increased by 3.2- and 8-fold in fatty streaks and atherosclerotic plaques, respectively, in comparison to healthy tissue (Andreeva et al. 1998). The highest content of (up to 25%) lipid-laden cells was found in fatty streaks located in the upper section of the proteoglycan-rich layer, encompassing almost two-thirds of it (Orekhov and Bobryshev 2015).

Thus, microscopic analysis of intimal thickness along the vessels affected with atherosclerosis, compared to normal tissues, revealed that proteoglycan-rich layer was 2- and 4-fold thicker in fatty streaks and atherosclerotic plaques, respectively (Orekhov et al. 2016b). In contrast, muscular-elastic layer remained intact in fatty streaks and was only slightly thickened in the atherosclerotic plaque areas (Orekhov et al. 2016b). Taken together, these observations clearly indicated that, governed by lipid and collagen accumulation, intima of atherosclerosis-affected arteries was abnormally and considerably thickened in the proteoglycan-rich layer. In addition, lipid and collagen accumulation determine the severity of lipidosis and fibrosis associated with atherosclerosis. Thickening of the proteoglycan-rich layer of the intima is the major cause of the arterial stenosis. Continuously growing plaque narrows the arteries leading to the severe ischaemia, which can have severe consequences. Even more dangerous is potential plaque rupture that in many cases can be lethal, leading to acute coronary syndrome, stroke and atherothrombosis (Chistiakov et al. 2015).

Besides, lipid accumulation triggers cellular expansion of the intimal cells. Comparative studies of grossly normal aortic intima and atherosclerosis-affected regions demonstrated that alterations of the proteoglycan-rich layer in the atherosclerotic plaque were associated with the increased cell count (Orekhov et al. 2016b).

According to the current knowledge, the increased cellularity in the proteoglycan-rich layer of atherosclerotic intima is caused by the recruitment of circulating immune cells and/or enhanced proliferation of the resident intimal cells (pericytes). The highest number of cells was detected in lipofibrous plaques. Intimal resident cells account for a majority of the intimal cell population (84-93%), therefore, the rise in their number determines the increase in the cellularity of atherosclerotic lesions. Nevertheless, the level of resident intimal cell proliferation is variable depending on plaque developmental stage and location (Orekhov et al. 2016b). Thus, the amount of resident proliferating cells in the lipid-laden atherosclerotic lesions, i.e. the early-stage lesions (fatty streak and fibrolipid plaques), was 10- to 20-fold greater than in uninvolved intima. Also, the number of resident proliferating cells in fibrous plaques, which are the later stage lesions, was detected to be lower than in lipid-enriched lesions, but considerably higher than in uninvolved intima (Orekhov et al. 1998). Moreover, the inflammatory cell proliferation is also present in the vascular wall; however, the proliferative index of inflammatory cells does not change in atherosclerotic lesions. Apparently, increased number of the inflamma-

tory cells is a result of their migration into subendothelial intima from the circulation. In contrast to pericytes, proliferative activity of inflammatory cells is not stimulated in atherosclerosis (Orekhov et al. 2010). Hence, these findings indicated that the increase in cellularity of the arterial intima is a result of proliferative 'splash' of resident cells in lipid-rich lesions (fatty streaks and fibrolipid plaques) and the migration of the inflammatory cells. Additionally, robust data confirming the close correlation between the number of pericytes and the content of intimal lipids were obtained. The correlation coefficients between the number of pericyte-like cells and collagen content, and intimal thickness were more significant than between other cell types of the intima (Orekhov and Bobryshev 2015). Relying on the findings that proliferating pericytes to a large extent are responsible for the increased cellularity and also are associated with lipid and collagen accumulation in the atherosclerosis affected site, they were suggested to be the key cells driving the process of intimal medial thickness, and, consequently, the growth of atherosclerotic lesion.

From this point of view, the main manifestations of atherosclerosis are accompanied by the following changes on the cellular level: lipid accumulation leading to the development of foam cells, local increase in the cell count as a result of cell proliferation and migration, accumulation of connective tissue matrix, and disruption of intercellular communication. These functional alterations of intimal cells were shown to be related to the stages of atherosclerotic lesion development. Thus, as described by Orekhov et al. (2016b), lipidosis, proliferative activity and collagen synthesis were presented in a bell-shaped manner. Proliferative activity and collagen accumulation were increased from a low or even zero level in uninvolved intima to the maximum in lipid-rich lesions (fatty streak and atheroma), but declined in fibrous plaques. Cellular lipidosis was gradually increased from uninvolved intima to fibrolipid plaques and then decreased in fibrous plaques, becoming lower than in uninvolved intima. Notably, the intracellular communication rate was on a constant decrease along the lesion progression, down to the zero level in fibrous plaques, where the cellular network was disrupted, compared to uninvolved intima, where integrity of cell network was maintained.

Furthermore, it was demonstrated that lipid accumulation caused by circulating LDL can orchestrate pro-inflammatory environment in the arterial intima, stimulating both adaptive and innate immunity (Hartvigsen et al. 2009). Consequently, the atherosclerotic plaque growth is linked to the local inflammation. Microscopic studies made it evident that upon lipid accumulation, pericytes, along with macrophages and dendritic cells, express antigen-presenting complexes (APC) and that is indicative of their involvement in the antigen presentation and the inflammatory progression in the arterial wall (Ivanova et al. 2015) (Fig. 13.2).

Thus, a population of stellate subendothelial cells expressing HLA-DR component of the major histocompatibility complex class II (MHC II), secreting pro-inflammatory cytokines and chemokines positively correlated with a number of immune-inflammatory cells in the atherosclerotic lesion (Handunnetthi et al. 2010; Bobryshev et al. 2011; Psaltis and Simari 2015).

Apart from lipid accumulation, intimal thickening and inflammation, the processes that directly contribute to atherosclerotic lesion development, pericytes play

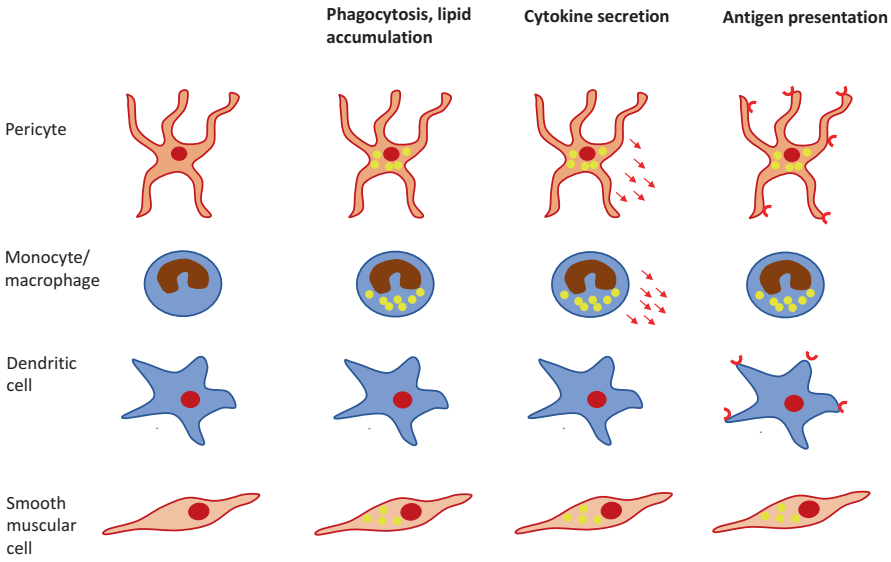

Fig. 13.2 Scheme is presenting the roles of arterial intimal cells in atherogenesis. Several types of arterial intimal cells participate in lipid accumulation and formation of foam cells. Macrophages and intimal pericytes accumulate lipids via their phagocytic activity and participate in the local inflammatory process secreting pro-inflammatory cytokines and chemokines. Dendritic cells, along with macrophages and intimal pericytes, express antigen-presenting complexes, further promoting the local inflammatory process. Note: World Journal of Cardiology. 2015;7(10):583–593. Doi: https://doi.org/10.4330/wjc.v7.i10.583

additional roles in this pathology, including neovascularization, plaque calcification and regulation of thrombogenesis. In atherosclerotic lesions, pericyte involvement in angiogenesis is mediated by upregulated expression of T-cadherin. This process is stimulated via activation of Erk1/2 tyrosine kinase pathway and NF-B nuclear translocation in pericytes, followed by the inhibition of adipogenic and activation of their chondrogenic differentiation pathways. Inhibition of adipogenic and stimulation of chondrogenic differentiation is regulated by Wnt/beta-catenin pathway (Kirton et al. 2007) and enhanced with transforming growth factor (TGF)-β3 expressed by macrophages, foam cells and vSMCs in the atherosclerotic plaque (Bobik 2006). Also, TGF-β3 signalling is associated with augmented glycosaminoglycan accumulation in the extracellular matrix during the development of fatty streaks and subsequent atheroma (Farrington-Rock et al. 2004).

Moreover, pericytes can be recruited to neovessels in atherosclerotic plaques via hepatocyte growth factor signalling activation triggering c-Met-PI3K/Akt pathway (Ivanova et al. 2015).

In addition, vascular calcification-associated factor expression was detected in the intentional pericytes promoting vascular calcification in the pro-inflammatory environment of the plaque (Ivanov et al. 2001; Takeuchi and Ohtsuki 2001).

At advanced stages of atherosclerotic plaque development, excessive expression of thrombogenic tissue factor by the subendothelial cells (pericytes) in the endothelium-uncovered arterial wall results in platelet aggregation and the formation of so-called fibrous cap covering the plaque (Ardissino et al. 2001; Orekhov et al. 2014). According to current understanding, fibrous cap has protective function, insulating the plaque from the blood flow. Its damage, however, can rapidly lead to thrombus formation, often with severe consequences.

Commentary on Likely Future Trends and Directions

Pericytes, as a part of cellular re-arrangements in the vascular wall, play a crucial role in atherosclerotic lesion formation. However, many questions about this enigmatic cell type remain unanswered. It would be important to further explore the relationship between MSC and pericytes in anticipation of unequivocal perceptions of pericyte origin. Furthermore, reliable pericyte markers or their combinations should be established. Such markers may serve as potential molecular targets in aid of diagnosis and therapeutic strategies in atherosclerosis and other vascular pathologies. Moreover, pericytes remain interesting potential points of therapeutic intervention, since they likely orchestrate the key atherosclerosis-related processes at the local tissue level, including local inflammation, plaque development, calcification and fibrosis, and further plaque growth accompanied by neovascularization.

Acknowledgment *Funding*: The research was funded by Russian Science Foundation (Grant no. 19-15-00010).

References

Al Ahmad, A., Taboada, C. B., Gassmann, M., & Ogunshola, O. O. (2011). Astrocytes and pericytes differentially modulate blood-brain barrier characteristics during development and hypoxic insult. *Journal of Cerebral Blood Flow and Metabolism, 31*(2), 693–705. https://doi.org/10.1038/jcbfm.2010.148.

Andreeva, E. R., Serebryakov, V. N., & Orekhov, A. N. (1995). Gap junctional communication in primary culture of cells derived from human aortic intima. *Tissue and Cell, 27*(5), 591–597.

Andreeva, E. R., Pugach, I. M., Gordon, D., & Orekhov, A. N. (1998). Continuous subendothelial network formed by pericyte-like cells in human vascular bed. *Tissue and Cell, 30*(1), 127–135.

Andreeva, E. R., Orekhov, A. N., & Smirnov, V. N. (1991). Quantitative estimation of lipid-laden cells in atherosclerotic lesions of the human aorta. *Acta Anatomica (Basel), 141*, 316–323.

Andreeva, E. R., Pugach, I. M., & Orekhov, A. N. (1997a). Collagen-synthesizing cells in initial and advanced atherosclerotic lesions of human aorta. *Atherosclerosis, 130*, 133–142.

Andreeva, E. R., Pugach, I. M., & Orekhov, A. N. (1997b). Subendothelial smooth muscle cells of human aorta express macrophage antigen in situ and in vitro. *Atherosclerosis, 135*, 19–27.

Armulik, A., Abramsson, A., & Betsholtz, C. (2005). Endothelial/pericyte interactions. *Circulation Research, 97*, 512–523.

Armulik, A., Genové, G., & Betsholtz, C. (2011). Pericytes: Developmental, physiological, and pathological perspectives, problems, and promises. *Developmental Cell, 21*(2), 193215.

Ardissino, D., Merlini, P. A., Bauer, K. A., Bramucci, E., Ferrario, M., Coppola, R., Fetiveau, R., Lucreziotti, S., Rosenberg, R. D., & Mannucci, P. M. (2001). Thrombogenic potential of human coronary atherosclerotic plaques. *Blood, 98*(9), 2726–2729. https://doi.org/10.1182/blood.V98.9.2726.

Balabanov, R., & Dore-Duffy, P. (1998). Role of the CNS microvascular pericyte in the blood-brain barrier. *Journal of Neuroscience Research, 53*, 637–644. https://doi.org/10.1002/(SICI)1097-4547(19980915)53:6<637::AID-JNR1>3.0.CO;2-6.

Bandopadhyay, R., Orte, C., Lawrenson, J. G., Reid, A. R., De Silva, S., & Allt, G. (2001). Contractile proteins in pericytes at the blood-brain and blood-retinal barriers. *Journal of Neurocytology, 30*(1), 35–44.

Bergers, G., & Song, S. (2005). The role of pericytes in blood-vessel formation and maintenance. *Neuro-Oncology, 7*(4), 452–464. https://doi.org/10.1215/S1152851705000232.

Betsholtz, C., Lindblom, P., & Gerhardt, H. (2005). Role of pericytes in vascular morphogenesis. *EXS, 94*, 5–125.

Birbrair, A., Zhang, T., Wang, Z.-M., et al. (2013). Role of pericytes in skeletal muscle regeneration and fat accumulation. *Stem Cells and Development, 22*(16), 2298–2314. https://doi.org/10.1089/scd.2012.0647.

Birbrair, A., Zhang, T., Wang, Z.-M., Messi, M. L., Mintz, A., & Delbono, O. (2015). Pericytes at the intersection between tissue regeneration and pathology. *Clinical Science (London, England), 128*(2), 81–93.

Bobik, A. (2006). Transforming growth factor-βs and vascular disorders. *Arteriosclerosis, Thrombosis, and Vascular Biology, 26*, 1712–1720. https://doi.org/10.1161/01.ATV.0000225287.20034.2c.

Bobryshev, Y. V., & Lord, R. S. (2000). CD1 expression and the nature of CD1-expressing cells in human atherosclerotic plaques. The American journal of pathology, 156(4), 1477–1478.

Bobryshev, Y. V., Andreeva, E. R., Mikhailova, I. A., Andrianova, I. V., Moisenovich, M. M., Khapchaev, S., et al. (2011). Correlation between lipid deposition, immune-inflammatory cell content and MHC class II expression in diffuse intimal thickening of the human aorta. *Atherosclerosis, 219*(1), 171–183.

Bose, A., Barik, S., Banerjee, S., et al. (2013). Tumor-derived vascular pericytes anergize Th cells. *Journal of Immunology, 191*(2), 971. https://doi.org/10.4049/jimmunol.1300280.

Bostrom, K., Watson, K. E., Horn, S., Wortham, C., Herman, I. M., & Demer, L. L. (1993). Bone morphogenetic protein expression in human atherosclerotic lesions. *The Journal of Clinical Investigation, 91*, 1800–1809.

Bouacida, A., Rosset, P., Trichet, V., et al. (2012). Pericyte-like progenitors show high immaturity and engraftment potential as compared with mesenchymal stem cells. *PLoS One, 7*(11), e4864.

Bouchard, B. A., Shatos, M. A., & Tracy, P. B. (1997). Human brain pericytes differentially regulate expression of procoagulant enzyme complexes comprising the extrinsic pathway of blood coagulation. *Arteriosclerosis, Thrombosis, and Vascular Biology, 17*, 1–9. https://doi.org/10.1161/01.ATV.17.1.1.

Campagnolo, P., Cesselli, D., Al Haj Zen, A., Beltrami, A. P., Kränkel, N., Katare, R., Angelini, G., Emanueli, C., & Madeddu, P. (2010). Human adult vena saphena contains perivascular progenitor cells endowed with clonogenic and proangiogenic potential. *Circulation, 121*, 1735–1745.

Chen, J., Luo, Y., Hui, H., et al. (2017). CD146 coordinates brain endothelial cell-pericyte communication for blood–brain barrier development. *Proceedings of the National Academy of Sciences of the United States of America, 114*(36), E7622 LPE7631.

Chen, R. R., Silva, E. A., Yuen, W. W., et al. (2007). Spatio-temporal VEGF and PDGF delivery patterns blood vessel formation and maturation. *Pharmaceutical Research, 24*, 258. https://doi.org/10.1007/s11095-006-9173-4.

Chen, M. J., Yokomizo, T., Zeigler, B. M., Dzierzak, E., & Speck, N. A. (2009). Runx1 is required for the endothelial to haematopoietic cell transition but not thereafter. *Nature, 457*, 887–891.

Chistiakov, D. A., Orekhov, A. N., & Bobryshev, Y. V. (2015). Contribution of neovascularization and intraplaque haemorrhage to atherosclerotic plaque progression and instability. *Acta Physiologica, 213*, 539–553. https://doi.org/10.1111/apha.12438.

Cochain, C., & Zernecke, A. (2015). Macrophages and immune cells in atherosclerosis: Recent advances and novel concepts. *Basic Research in Cardiology, 110*(4), 34.

Collett, G. D. M., & Canfield, A. E. (2005). Angiogenesis and pericytes in the initiation of ectopic calcification. *Circulation Research, 96*, 930–938. https://doi.org/10.1161/01.RES.0000163634.51301.0d.

Corselli, M., Chen, C.-W., Sun, B., Yap, S., Rubin, J. P., & Péault, B. (2012). The tunica adventitia of human arteries and veins as a source of mesenchymal stem cells. *Stem Cells and Development, 21*(8), 1299–1308. https://doi.org/10.1089/scd.2011.0200.

Crisan, M., Zheng, B., Zambidis, E. T., Yap, S., Tavian, M., Sun, B., Giacobino, J. P., Casteilla, L., Huard, J., & Péault, B. (2007). Blood vessels as a source of progenitor cells in human embryonic and adult life. In N. M. Bilko, B. Fehse, W. Ostertag, C. Stocking, & A. R. Zander (Eds.), *Stem cells and their potential for clinical application, NATO Security through Science Series (A: Chemistry and Biology)* (pp. 137–147). Netherlands: Springer.

Crisan, M., Yap, S., Casteilla, L., et al. (2008). A perivascular origin for mesenchymal stem cells in multiple human organs. *Cell Stem Cell, 3*(3), 301–313. https://doi.org/10.1016/j.stem.2008.07.003.

Cuevas, P., Gutierrez-Diaz, J. A., Reimers, D., Dujovny, M., Diaz, F. G., & Ausman, J. I. (1984). Pericyte endothelial gap junctions in human cerebral capillaries. *Anatomy and Embryology, 170*, 155–159. https://doi.org/10.1007/BF00319000.

Dohgu, S., Takata, F., Yamauchi, A., Nakagawa, S., Egawa, T., Naito, M., Tsuruo, T., Sawada, Y., Niwa, M., & Kataoka, Y. (2005). Brain pericytes contribute to the induction and up-regulation of blood-brain barrier functions through transforming growth factor-beta production. *Brain Research, 1038*, 208–215. https://doi.org/10.1016/j.brainres.2005.01.027.

Dominici, M., Le Blanc, K., Mueller, I., Slaper-Cortenbach, I., Marini, F., Krause, D., Deans, R., Keating, A., Prockop, D. J., & Horwitz, E. (2006). Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy, 8*(4), 315–317.

Dondossola, E., Rangel, R., Guzman-Rojas, L., et al. (2013). CD13-positive bone marrow-derived myeloid cells promote angiogenesis, tumor growth, and metastasis. *Proceedings of the National Academy of Sciences of the United States of America, 110*(51), 20717–20722.

Dore-Duffy, P., & Cleary, K. (2011). Morphology and properties of pericytes. In S. Nag (Ed.), *The Blood-Brain and Other Neural Barriers. Methods in Molecular Biology (Methods and Protocols)* (p. 686). New York: Humana Press.

Dulmovits, B. M., & Herman, I. M. (2012). Microvascular remodeling and wound healing: a role for pericytes. *The International Journal of Biochemistry & Cell Biology, 44*(11), 1800–1812. https://doi.org/10.1016/j.biocel.2012.06.031.

Farrington-Rock, C., Crofts, N. J., Doherty, M. J., Ashton, B. A., Griffin-Jones, C., & Canfield, A. E. (2004). Chondrogenic and adipogenic potential of microvascular pericytes. *Circulation, 110*(15), 2226–2232.

Favero, G., Paganelli, G., Buffoli, B., Rodella, L. F., & Rezzani, R. (2014). Endothelium and its alterations in cardiovascular diseases: Life style intervention. *BioMed Research International, 2014*, 801896. https://doi.org/10.1155/2014/801896.

Gerhardt, H., & Betsholtz, C. (2003). Endothelial-pericyte interactions in angiogenesis. *Cell and Tissue Research, 314*, 15. https://doi.org/10.1007/s00441-003-0745-x.

Glentis, V., Gurchenkov, D., & Vignjevic, M. (2014). Assembly, heterogeneity, and breaching of the basement membranes. *Cell Adhesion & Migration, 8*, 236–245. https://doi.org/10.4161/cam.28733.

Groschner, L. N., Waldeck-Weiermair, M., Malli, R., et al. (2012). Endothelial mitochondria—less respiration, more integration. *Pflugers Archiv—European Journal of Physiology, 464*, 63. https://doi.org/10.1007/s00424-012-1085-z.

Guillemin, G. J., & Brew, B. J. (2004). Microglia, macrophages, perivascular macrophages, and pericytes: a review of function and identification. *Journal of Leukocyte Biology, 75*(3), 388–397. https://doi.org/10.1189/jlb.0303114.

Hall, C. N., Reynell, C., Gesslein, B., Hamilton, N. B., Mishra, A., Sutherland, B. A., & Attwell, D. (2014). Capillary pericytes regulate cerebral blood flow in health and disease. *Nature, 508*(7494), 55.

Handunnetthi, L., Ramagopalan, S. V., Ebers, G. C., & Knight, J. C. (2010). Regulation of major histocompatibility complex class II gene expression, genetic variation and disease. *Genes and Immunity, 11*, 99–112.

Hartvigsen, K., Chou, M. Y., Hansen, L. F., Shaw, P. X., Tsimikas, S., Binder, C. J., & Witztum, J. L. (2009). The role of innate immunity in atherogenesis. *Journal of Lipid Research, 50*(Suppl), S388–S393.

Haurani, M. J., & Pagano, P. J. (2007). Adventitial fibroblast reactive oxygen species as autacrine and paracrine mediators of remodeling: Bellwether for vascular disease? *Cardiovascular Research, 75*(4), 679–668.

Hellstrom, M., Gerhardt, H., Kalen, M., Li, X., Eriksson, U., Wolburg, H., & Betsholtz, C. (2001). Lack of pericytes leads to endothelial hyperplasia and abnormal vascular morphogenesis. *The Journal of Cell Biology, 153*, 543–553.

Herman, I. M. (2010). Pericytes and microvascular remodeling: Regulation of retinal angiogenesis. In D. A. Dartt (Ed.), *Encyclopedia of the Eye* (pp. 296–299). Oxford: Academic Press. https://doi.org/10.1016/B978-0-12-374203-2.00149-4.

Hughes, S., Gardiner, T., Hu, P., Baxter, L., Rosinova, E., & Chan-Ling, T. (2006). Altered pericyte-endothelial relations in the rat retina during aging: implications for vessel stability. *Neurobiology of Aging, 27*, 1838–1847.

Ivanov, D., Philippova, M., Antropova, J., et al. (2001). Expression of cell adhesion molecule T-cadherin in the human vasculature. *Histochemistry and Cell Biology, 3*(115), 231–242.

Ivanova, E. A., Bobryshev, Y. V., & Orekhov, A. N. (2015). Intimal pericytes as the second line of immune defence in atherosclerosis. *World Journal of Cardiology, 7*(10), 583–593. https://doi.org/10.4330/wjc.v7.i10.583.

Ivanova, E. A., & Orekhov, A. N. (2016). Cellular model of atherogenesis based on pluripotent vascular wall pericytes. *Stem Cells International, 2016*, 7321404. https://doi.org/10.1155/2016/7321404.

Juchem, G., Weiss, D. R., Gansera, B., Kemkes, B. M., Mueller-Hoecker, J., & Nees, S. (2010). Pericytes in the macrovascular intima: possible physiological and pathogenetic impact. *The American Journal of Physiology-Heart and Circulatory Physiology, 3*(298), 754–770.

Kim, J. A., Tran, N. D., Li, Z., Yang, F., Zhou, W., & Fisher, M. J. (2006). Brain endothelial hemostasis regulation by pericytes. *Journal of Cerebral Blood Flow and Metabolism, 26*, 209–217. https://doi.org/10.1038/sj.jcbfm.9600181.

Kirton, J. P., Wilkinson, F. L., Canfield, A. E., & Alexander, M. Y. (2006). Dexamethasone downregulates calcification-inhibitor molecules and accelerates osteogenic differentiation of vascular pericytes: Implications for vascular calcification. *Circulation Research, 98*(10), 1264–1272.

Kirton, P., Crofts, N. J., George, S. J., Brennan, K., & Canfield, A. E. (2007). Wnt/T-catenin signaling stimulates chondrogenic and inhibits adipogenic differentiation of pericytes: potential relevance to vascular disease? *Circulation Research, 6*(101), 581–589.

Koh, W., Stratman, A. N., Sacharidou, A., & Davis, G. E. (2008). *In vitro* three dimensional collagen matrix models of endothelial lumen formation during vasculogenesis and angiogenesis. *Methods in Enzymology, 443*, 83–101. https://doi.org/10.1016/S0076-6879(08)02005-3.

Korn, J., Christ, B., & Kurz, H. (2002). Neuroectodermal origin of brain pericytes and vascular smooth muscle cells. *The Journal of Comparative Neurology, 442*, 78–88. https://doi.org/10.1002/cne.1423.

Kostallari, E., Baba-Amer, E., Alonso-Martin, S., Ngoh, P., Relaix, F., Lafuste, P., & Gherardi, R. K. (2015). Pericytes in the myovascular niche promote post-natal myofiber growth and satellite cell quiescence. *Development, 142*, 1242–1253. https://doi.org/10.1242/dev.115386.

Kovacic, J. C., & Boehm, M. (2009). Resident vascular progenitor cells: An emerging role for non terminally differentiated vessel-resident cells in vascular biology. *Stem Cell Research, 2*(1), 2–15.

Krueger, M., & Bechmann, I. (2010). CNS pericytes: Concepts, misconceptions, and a way out. *Glia, 58*, 1–10. https://doi.org/10.1002/glia.20898.

Leveen, P., Pekny, M., Gebre-Medhin, S., Swolin, B., Larsson, E., & Betsholtz, C. (1994). Mice deficient for PDGF B show renal, cardiovascular, and hematological abnormalities. *Genes & Development, 8*, 1875–1887.

Li, X., Xiao, Y., Cui, Y., Tan, T., Narasimhulu, C. A., Hao, H., Liu, L., Zhang, J., He, G., Verfaillie, C. M., Lei, M., Parthasarathy, S., Ma, J., Zhu, H., & Liu, Z. (2014). Cell membrane damage is involved in the impaired survival of bone marrow stem cells by oxidized low-density lipoprotein. *Journal of Cellular and Molecular Medicine, 18*, 2445–2453. https://doi.org/10.1111/jcmm.12424.

Lindahl, P., Johansson, B. R., Leveen, P., & Betsholtz, C. (1997). Pericyte loss and microaneurysm formation in PDGF-B-deficient mice. *Science, 277*, 242–245.

Mazanet, R., & Franzini-Armstrong, C. (1982). Scanning electron microscopy of pericytes in rat red muscle. *Microvascular Research, 23*(3), 361–369.

Mollace, V., Gliozzi, M., Musolino, V., Carresi, C., Muscoli, S., Mollace, R., Tavernese, A., Gratteri, S., Palma, E., Morabito, C., Vitale, C., Muscoli, C., Fini, M., & Romeo, F. (2015). Oxidized LDL attenuates protective autophagy and induces apoptotic cell death of endothelial cells: Role of oxidative stress and LOX-1 receptor expression. *International Journal of Cardiology, 184*, 152–158.

Montiel-Eulefi, E., Nery, A. A., Rodrigues, L. C., Sánchez, R., Romero, F., & Ulrich, H. (2012). Neural differentiation of rat aorta pericyte cells. *Cytometry, 81A*, 65–71. https://doi.org/10.1002/cyto.a.21152.

Moreno, P. R., Purushothaman, M., & Purushothaman, K. R. (2012). Plaque neovascularization: Defense mechanisms, betrayal, or a war in progress. *Annals of the New York Academy of Sciences, 1254*, 7–17.

Morikawa, S., Baluk, P., Kaidoh, T., Haskell, A., Jain, R. K., & McDonald, D. M. (2002). Abnormalities in pericytes on blood vessels and endothelial sprouts in tumors. *The American Journal of Pathology, 160*(3), 985–1000.

Moore, K. J., & Tabas, I. (2011). Macrophages in the pathogenesis of atherosclerosis. *Cell, 145*(3), 341–355.

Mozaffarian, D., Benjamin, E. J., Go, A. S., Arnett, D. K., Blaha, M. J., Cushman, M., de Ferranti, S., Després, J. P., Fullerton, H. J., Howard, V. J., Huffman, M. D., Judd, S. E., Kissela, B. M., Lackland, D. T., Lichtman, J. H., Lisabeth, L. D., Liu, S., Mackey, R. H., Matchar, D. B., DK, M. G., Mohler, E. R., Moy, C. S., Muntner, P., Mussolino, M. E., Nasir, K., Neumar, R. W., Nichol, G., Palaniappan, L., Pandey, D. K., Reeves, M. J., Rodriguez, C. J., Sorlie, P. D., Stein, J., Towfighi, A., Turan, T. N., Virani, S. S., Willey, J. Z., Woo, D., Yeh, R. W., Turner, M. B., & American Heart Association Statistics Committee and Stroke Statistics Subcommittee. (2015). Heart disease and stroke statistics-2015 update: A report from the American Heart Association. *Circulation, 131*, e29–e322.

Murray, I. R., West, C. C., Hardy, W. R., et al. (2014). Natural history of mesenchymal stem cells, from vessel walls to culture vessels. *Cellular and Molecular Life Sciences, 71*, 1353. https://doi.org/10.1007/s00018-013-1462-6.

Nakata, M., Nakagomi, T., Maeda, M., et al. (2017). Induction of perivascular neural stem cells and possible contribution to neurogenesis following transient brain ischemia/reperfusion injury. *Translational Stroke Research, 8*, 131. https://doi.org/10.1007/s12975-016-0479-1.

Nayak, R. C., Berman, A. B., George, K. L., Eusenbarth, G. S., & King, G. L. (1988). A monoclonal antibody (3G5)-defined ganglioside antigen is expressed on the cell surface of microvascular pericytes. *The Journal of Experimental Medicine, 4*, 1003–1015.

Nazarova, V. L., Andreeva, E. R., Tertov, V. V., Gel'dieva, B. S., & Orekhov, A. N. (1995). Immunocytochemical study to localize a scavenger receptor in human aorta smooth muscle cells. *Biulleten' Eksperimental'noĭ Biologii i Meditsiny, 120*, 195–198.

Nicosia, R. F. (2009). The aortic ring model of angiogenesis: a quarter century of search and discovery. *Journal of Cellular and Molecular Medicine, 13*(10), 4113–4136.

Oberlin, E., Tavian, M., Blazsek, I., & Peault, B. (2002). Blood-forming potential of vascular endothelium in the human embryo. *Development, 129*, 4147–4157.

Orekhov, A. N., Andreeva, E. R., Krushinsky, A. V., Novikov, I. D., Tertov, V. V., Nestaiko, G. V., Khashimov, K. A., Repin, V. S., & Smirnov, V. N. (1986). Intimal cells and atherosclerosis. Relationship between the number of intimal cells and major manifestations of atherosclerosis in the human aorta. *The American Journal of Pathology, 125*, 402–415.

Orekhov, A. N., Andreeva, E. R., & Bobryshev, Y. V. (2016a). Cellular mechanisms of human atherosclerosis: Role of cell-to-cell communications in subendothelial cell functions. *Tissue & Cell, 48*(1), 25–34. https://doi.org/10.1016/j.tice.2015.11.002.

Orekhov, A. N., Andreeva, E. R., Ivanova, E. A., & Bobryshev, Y. V. (2016b). Role of pericytes and modified lipoprotein in human atherogenesis. In *Coronary Artery Disease—Research and Practice*. Kowloon: iConcept Press Ltd.

Orekhov, A. N., Bobryshev, Y. V., & Chistiakov, D. A. (2014). The complexity of cell composition of the intima of large arteries: Focus on pericyte-like cells. *Cardiovascular Research, 103*, 438–451.

Orekhov, A. N., Andreeva, E. R., Andrianova, I. V., & Bobryshev, Y. V. (2010). Peculiarities of cell composition and cell proliferation in different type atherosclerotic lesions in carotid and coronary arteries. *Atherosclerosis, 212*(2), 436–443.

Orekhov, A. N., Andreeva, E. R., Mikhailova, I. A., & Gordon, D. (1998). Cell proliferation in normal and atherosclerotic human aorta: proliferative splash in lipid-rich lesions. *Atherosclerosis, 139*(1), 41–48.

Orekhov AN and Bobryshev YV. (2015). Cell composition of the subendothelial aortic intima and the role of alpha-smooth muscle actin expressing pericyte-like cells and smooth muscle cells in the development of atherosclerosis. Tissue Engineering and Regenerative Medicine. Muscle Cell and Tissue. Chapter 8. Doi: https://doi.org/10.5772/60430.

Pallone, T. L., Silldorff, E. P., & Turner, M. R. (1998). Intrarenal blood flow: microvascular anatomy and the regulation of medullary perfusion. *Clinical and Experimental Pharmacology & Physiology, 25*, 383–392.

Pallone, T. L., Zhang, Z., & Rhinehart, K. (2003). Physiology of the renal medullary microcirculation. *American Journal of Physiology. Renal Physiology, 284*, 253–266.

Pantakani, D. V. K., & Asif, A. (2015). Atherosclerosis: Epigenetic targeting of macrophages in disease management. *Journal of Clinical & Cellular Immunology, 6*, e118. https://doi.org/10.4172/2155-9.

Peppiatt, C. M., Howarth, C., Mobbs, P., & Attwell, D. (2006). Bidirectional control of CNS capillary diameter by pericytes. *Nature, 443*(7112), 700–704. https://doi.org/10.1038/nature05193.

Psaltis, J., & Simari, A. D. (2015). Vascular wall progenitor cells in health and disease. *Circulation Research, 8*(1166), 1392–1412.

Rajamannan, N. M., Subramaniam, M., Rickard, D., Stock, S. R., Donovan, J., Springett, M., Orszulak, T., Fullerton, D. A., Tajik, A. J., Bonow, R. O., & Spelsberg, T. (2003). Human aortic valve calcification is associated with an osteoblast phenotype. *Circulation, 107*, 2181–2184.

Raza, A., Franklin, M. J., & Dudek, A. Z. (2010). Pericytes and vessel maturation during tumor angiogenesis and metastasis. *American Journal of Hematology, 85*, 593–598. https://doi.org/10.1002/ajh.21745.

Rekhter, M. D., Tertov, V. V., Andreeva, E. R., Kolpakov, V. A., Mironov, A. A., & Orekhov, A. N. (1993). Lipid accumulation in the subendothelial cells of human aortic intima impairs cell-to-cell contacts: A comparative study in situ and in vitro. *Cardiovascular Pathology, 2*(1), 53–62.

Rossi, E., Smadja, D. M., Boscolo, E., et al. (2016). Endoglin regulates mural cell adhesion in the circulatory system. *Cellular and Molecular Life Sciences, 73*, 1715–1739. https://doi.org/10.1007/s00018-015-2099-4.

Rucker, H. K., Wynder, H. J., & Thomas, W. E. (2000). Cellular mechanisms of CNS pericytes. *Brain Research Bulletin, 51*, 363–369.

Salvayre, R., Auge, N., Benoist, H., & Negre-Salvayre, A. (2002). Oxidized low-density lipoprotein-induced apoptosis. *Biochimica et Biophysica Acta, 1585*(2–3), 213–221.

Shashkin, P., Dragulev, B., & Ley, K. (2005). Macrophage differentiation to foam cells. *Current Pharmaceutical Design, 11*(23), 3061–3072.

Sims, D. E. (2000). Diversity Within Pericytes. *Clinical and Experimental Pharmacology and Physiology, 27*, 842–846. https://doi.org/10.1046/j.1440-1681.2000.03343.x.

Song, L., Lee, C., & Schindler, C. (2011). Deletion of the murine scavenger receptor CD68. *Journal of Lipid Research, 52*, 1542–1550.

Steitz, S. A., Speer, M. Y., Curinga, G., et al. (2001). Smooth muscle cell phenotypic transition associated with calcification. *Circulation Research, 89*(12), 1147–1154.

Stefanska, A. (2013). Renal pericytes: Multifunctional cells of the kidneys. *Pflugers Archiv—European Journal of Physiology, 465*, 767–773. https://doi.org/10.1007/s00424-013-1263-7.

Tabas, I., García-Cardeña, G., & Owens, G. K. (2015). Recent insights into the cellular biology of atherosclerosis. *The Journal of Cell Biology, 209*(1), 13–22. https://doi.org/10.1083/jcb.201412052.

Takeuchi, T., & Ohtsuki, Y. (2001). Recent progress in T-cadherin (CDH13, H-cadherin) research. *Histology and Histopathology, 4*(16), 1287–1293.

Tintut, Y., Alfonso, Z., Saini, Z., Radcliff, K., Watson, K., & Boström Kand Demer, L. L. (2003). Multilineage potential of cells from the artery wall. *Circulation, 108*, 2505–2510. https://doi.org/10.1161/01.CIR.0000096485.64373.C5.

Thomas, W. E. (1999). Brain macrophages: On the role of pericytes and perivascular cells. *Brain Research Reviews, 31*(1), 42–57.

van Dijk, C. G., Nieuweboer, F. E., Pei, J. Y., Xu, Y. J., Burgisser, P., van Mulligen, E., el Azzouzi, H., Duncker, D. J., Verhaar, M. C., & Cheng, C. (2015). The complex mural cell: Pericyte function in health and disease. *International Journal of Cardiology, 190*, 75–89.

Wang, P. C., Vancura, A., Mitcheson, T. G., & Kuret, J. (1992). Two genes in *Saccharomyces cerevisiae* encode a membrane-bound form of casein kinase-1. *Molecular Biology of the Cell, 3*(3), 275–286.

Warmke, N., Griffin, K. J., & Cubbon, R. M. (2016). Pericytes in diabetes-associated vascular disease. *Journal of Diabetes and its Complications, 8*(30), 1643–1650. https://doi.org/10.1016/j.jdiacomp.2016.08.005.

Wu, D. M., Kawamura, H., Sakagami, K., Kobayashi, M., & Puro, D. G. (2003). Cholinergic regulation of pericyte-containing retinal microvessels. *American Journal of Physiology-Heart and Circulatory Physiology, 284*(6), H2083–H2090.

Yemisci, M., Gursoy-Ozdemir, Y., Vural, A., Can, A., Topalkara, K., & Dalkara, T. (2009). Pericyte contraction induced by oxidative-nitrative stress impairs capillary reflow despite successful opening of an occluded cerebral artery. *Nature Medicine, 15*(9), 1031.

Zambidis, E. T., Oberlin, E., Tavian, M., & Peault, B. (2006). Blood-forming endothelium in human ontogeny: Lessons from in utero development and embryonic stem cell culture. *Trends in Cardiovascular Medicine, 16*, 95–101.

Zheng, B., Cao, B., Crisan, M., Sun, B., Li, G. H., Logar, A., et al. (2007). Prospective identification of myogenic endothelial cells in human skeletal muscle. *Nature Biotechnology, 25*(9), 1025–1034.