Chapter 12

The Role of Vascular Smooth Muscle Cells in the Physiology and Pathophysiology of Blood Vessels

Lucie Bacakova, Martina Travnickova, Elena Filova, Roman Matějka, Jana Stepanovska, Jana Musilkova, Jana Zarubova and Martin Molitor

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.77115

Abstract

Vascular smooth muscle cells (VSMCs) play important roles not only in the physiological functions of the blood vessels, such as vasoconstriction, vasodilatation and extracellular matrix production, but also in the pathogenesis of vascular diseases, particularly atherosclerosis and hypertension. VSMCs are mostly of mesodermal origin, although some are of neuroectodermal origin, for example, VSMCs present in the aorta and in blood vessels arising from the aortic arch. VSMCs of neuroectodermal origin are implicated in defects of cardiovascular morphogenesis, such as bicuspid aortic valve, coarctation of the aorta, patent ductus arteriosus and tetralogy of Fallot. The origin, location in the vascular tree, gender, species, strain and age influence the phenotype of VSMCs and their propensity to migration and growth. In a healthy adult organism, VSMCs have a quiescent and differentiated contractile phenotype characterized by early markers (e.g., SM α-actin, SM22-α), intermediate markers (h-caldesmon, calponin) and late markers (SM myosins, smoothelin) of VSMC differentiation. However, after blood vessel injury, surgery or explantation *in vitro*, VSMCs undergo a phenotypic modulation to synthetic phenotype, which endows them with high activity in migration, growth and proteosynthesis. These features can lead to stenosis or to obliteration of the vascular lumen and impaired blood supply to various tissues and organs.

Keywords: blood vessels, smooth muscle cells, contractile phenotype, synthetic phenotype, phenotypic modulation, vascular diseases, atherosclerosis, hypertension, developmental pathology

1. Introduction

Vascular smooth muscle cells (VSMCs) are an important component of blood vessels. The cells are located in the medium part of a blood vessel, that is, *tunica media*, where they are oriented in a circle around the vascular lumen and form numerous layers. In medium vessels, there are up to 40 layers of VSMCs, and in large vessels, there are up to 60 layers. As the medium part of the blood vessel wall, the *tunica media* is located between the *tunica intima* and *tunica adventitia*, and is separated from these parts by the *lamina elastica interna* and the *lamina elastica externa*, respectively. The *tunica intima* (also referred to as *tunica interna*) contains a semipermeable monolayer of endothelial cells and forms the luminal part of a blood vessel, contacting the blood. The *tunica adventitia*, that is, the exterior part of a blood vessel (also referred to as *tunica externa*), contains fibroblasts, nerves and, in bigger vessels, also small blood vessels supplying the vascular wall, called *vasa vasorum*. In addition, the *tunica adventitia* anchors blood vessels to the adjacent tissues (**Figure 1**) [1]. This structure is similar in arteries and in veins; in veins, the *tunica media* is usually thinner, due to the lower blood pressure in the venous bed, and the *tunica intima* in some veins contains valves in order to keep blood flowing in a single direction (**Figure 2**) [2]. The smallest blood vessels are capillaries, which lack the three classical layers of a blood vessel wall. They consist only of a fine tubular structure built of endothelial cells, which is surrounded by pericytes, which are somewhat like VSMCs and are phenotypically similar to VSMCs (**Figure 3**) [3].

VSMCs play important roles in the physiological functioning of blood vessels and in pathological changes in them. In healthy blood vessels of an adult organism, VSMCs ensure that the blood vessels contract and relax, and in this way, they make a marked contribution to the regulation of blood circulation. In healthy adult blood vessels, VSMCs are in a quiescent nonproliferative phenotype, referred to as contractile phenotype. This phenotype is characterized by abundant contractile fibers containing VSMC-specific contractile proteins, such as α-isoform of actin and the SM-1 and SM-2 myosin heavy chain isoforms, and other specific proteins

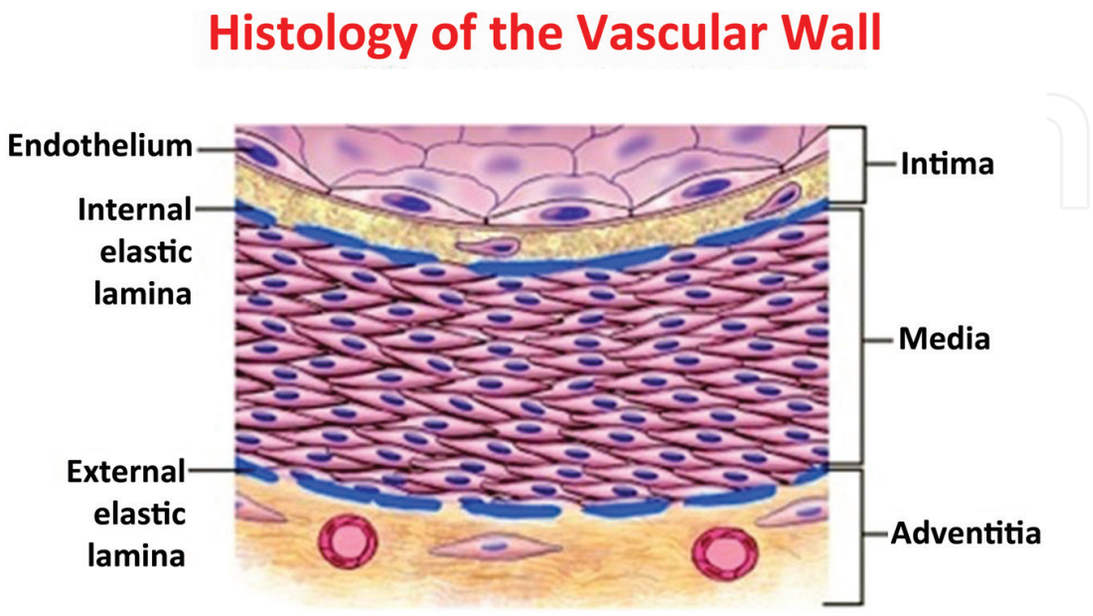

Figure 1. A scheme of the anatomy of an arterial wall. Available from [1].

http://dx.doi.org/10.5772/intechopen.77115

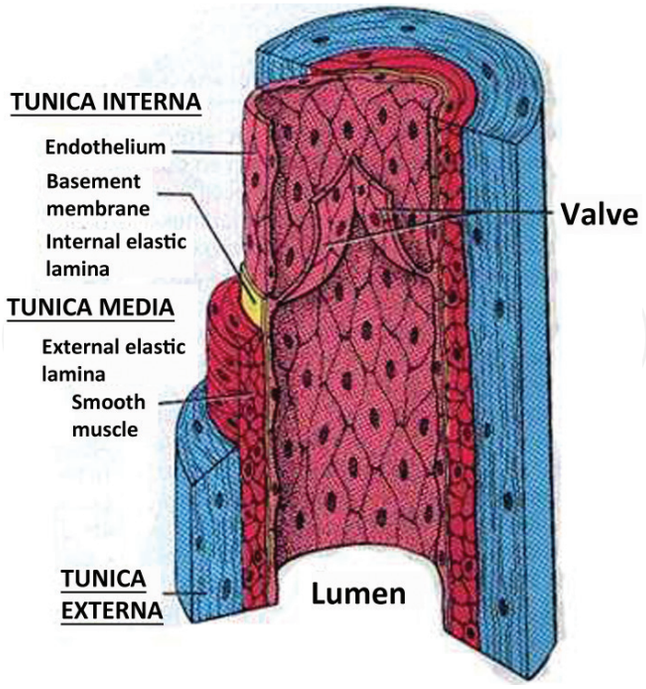

Figure 2. A scheme of the anatomy of a venous wall. Available from [2].

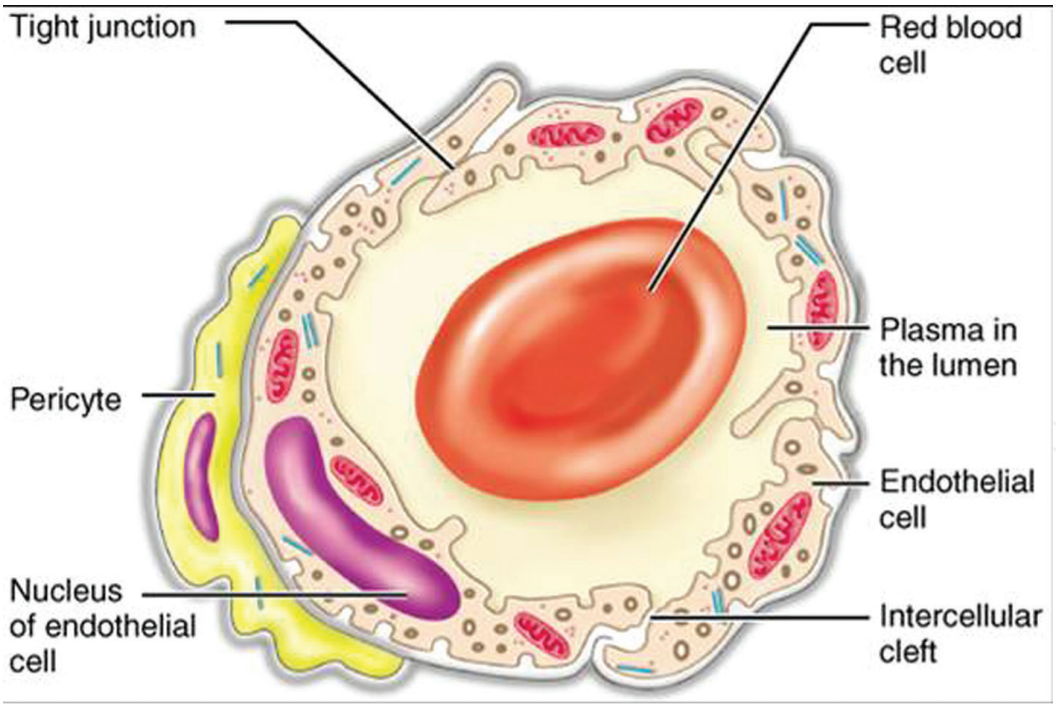

Figure 3. A scheme of the anatomy of a capillary. Available from [3].

associated with the contractile apparatus [4–8]. Under pathological conditions accompanying the onset and development of vascular diseases, VSMCs undergo a process referred to as phenotypic modulation, that is, they switch from the contractile phenotype to the synthetic phenotype, characterized by a loss of contractile filaments and associated molecules, and by increased formation of organelles associated with proteosynthesis (**Figure 4**) [9]. VSMCs of

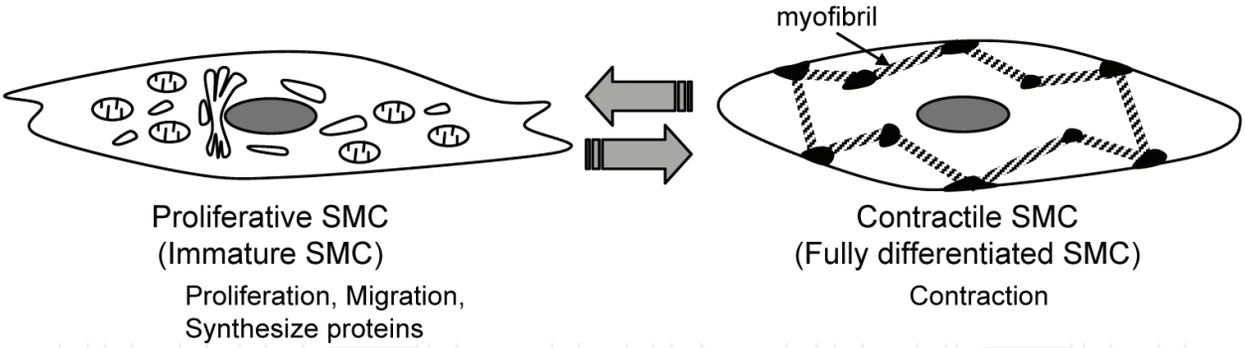

Figure 4. Transition between the contractile and synthetic phenotype in VSMC [9].

synthetic phenotype are active in migration and growth. This can lead to intimal thickening, formation of atherosclerotic plaques, thickening of the blood vessel wall during hypertension, and finally to stenosis or full obliteration of the vascular lumen [4–6, 9–11]. Similar changes in VSMCs also occur after vascular surgery and when VSMCs are cultivated *in vitro*, particularly under conventional static conditions and in standard serum-supplemented media [6, 7, 12].

This chapter summarizes the most important knowledge on the role of VSMCs in the physiological behavior and in pathological alterations of blood vessels, on the contractile and synthetic phenotype of VSMC, and on the implication of these cells in developmental disorders of the cardiovascular system, atherosclerosis and hypertension. This chapter includes the author's own experience in her studies of gender-related differences in the migration and proliferation of VSMCs, and in studying the role of VSMCs in vascular remodeling during hypoxic pulmonary hypertension.

2. Vascular smooth muscle cell origin and its role in vascular pathology

2.1. Sources of VSMCs

The VSMCs arise from two main sources: the mesoderm and the neuroectoderm, that is, the ectoderm of the neural crest. Most of the VSMCs in the vascular tree are of mesodermal origin, and are formed mainly from mesenchyme, that is, a type of connective tissue found mostly during the development of an embryo [13]; or from mesothelium *via* the epithelial-to-mesenchymal transition [14].

VSMCs differentiated from neural crest cells reside in the cardiac outflow tract, the ascending aorta, the aortic arch, the proximal thoracic aorta, the brachiocephalic trunks, the common carotid arteries, the internal and external carotid arteries and subclavian arteries, and also in the blood vessels of the facial structures and the forebrain [15–17]. The pericytes in regions supplied by these vessels are also of neuroectodermal origin [18]. Neural crest cells also differentiate into adventitial fibroblasts. However, the endothelial cells of all the vessels are of mesodermal origin [15, 19]. In addition, the coronary and pulmonary arteries and the descending aorta remain devoid of neuroectodermal VSMCs and contain only mesodermal VSMCs, similarly as the remaining vessels in the body [20].

The factors that regulate the differentiation of neural crest cells into VSMCs include the Notch and Hippo signaling pathways, fibronectin, transforming growth factor-β (TGF-β), Smad2, and myocardin-related transcription factor B.

The Notch plays a critical, cell-autonomous role in the differentiation of neural crest precursors into smooth muscle cells both *in vitro* and *in vivo.* Mutations in components of the Notch signaling pathway result in defects of the cardiac outflow tract [21]. For proper Notch signaling, Hippo signaling is required. Neural crest-specific deletion of the Hippo effectors Yap and Taz produces neural crest precursors that migrate normally, but fails to differentiate into VSMCs [22]. In addition, Notch signaling is regulated by fibronectin 1 (Fn1), which is synthesized by the neural crest cells (NCCs) and mediates the morphogenesis of the aortic arch artery. The Fn1 signals are delivered into NCCs through integrin $\alpha_5\beta_1$ adhesion receptors and lead to the differentiation of NCCs into VSMCs [23].

TGF-β plays a controversial role in VSMC differentiation. On the one hand, TGF-β induces the differentiation of VSMCs from NCCs. This differentiation is mediated by Smad2, a transcription factor which is required for TGF-β-induced nuclear translocation of myocardin-related transcription factor B (MRTFB). MRTFB enhanced the binding of Smad2 to a promoter of the expression of genes encoding differentiation markers of VSMCs [24, 25]. On the other hand, in mature VSMCs of neural crest origin, TGF-β increased the synthesis of DNA, which is known to be associated with a loss of differentiation markers in VSMCs. At the same time, TGF-β inhibited the growth of VSMCs of mesodermal origin. This dual effect of TGF-β was explained by the different composition of TGF-β receptors in VSMCs of different origin. In neuroectodermal VSMCs, subunit II is non-glycosylated, while in mesodermal VSMCs, subunit II of this receptor is fully glycosylated [26].

2.2. The role of VSMC origin in developmental pathology

Proper differentiation of neural crest cells into VSMCs is required for normal cardiovascular morphogenesis. If this differentiation is defective, various cardiovascular disorders can occur, for example, bicuspid aortic valve, coarctation of the aorta, patent ductus arteriosus, tetralogy of Fallot, aneurysm of the thoracic aorta, and intracranial aneurysm.

Bicuspid aortic valve (BAV) is associated with the decreased expression of MYH11, the gene encoding the myosin heavy chain in VSMCs of neural crest origin, impaired contraction of these cells and decreased TGF-β signaling based on phosphorylation of SMAD2. In addition, patients with BAV are at higher risk of developing aneurysms of the thoracic aorta than patients with tricuspid aortic valve [27].

The major role in the pathogenesis of coarctation of the aorta in humans is attributed to deregulation of the Forkhead Box C1 (FOXC1) transcription factor or its downstream genes [28]. FOXC1 is also involved in the pathogenesis of ocular diseases, particularly glaucoma. FOXC1 dysfunction causes disruptions in basement membrane integrity and lower resistance of cells to cell death in response to oxidative stress [29, 30]. In addition, patients with BAV and coarctation of the aorta are more prone to developing an intracranial aneurysm [31].

Patent ductus arteriosus, that is, a temporary fetal vessel that bypasses the lungs by shunting the aortic arch to the pulmonary artery, can result from insufficient proliferation, differentiation

and contractility of a specific smooth muscle subpopulation that shares a common neural crest precursor with cardiovascular melanocytes [32].

Tetralogy of Fallot (TOF) is a serious congenital disorder characterized by a ventricular septal defect, overriding aorta, right ventricular outflow tract obstruction (i.e., pulmonary stenosis) and right ventricular hypertrophy. TOF has a polygenic origin, being caused by a combination of deleterious mutations in genes essential for apoptosis and cell growth, for the assembly of the sarcomere, and also for the neural crest and the secondary heart field, that is, the cellular basis of the right ventricle and its outflow tract. Numerous genetic abnormalities are associated with TOF. They include mutation of the gene encoding myosin binding protein C3 (MYBPC3) [33], mutations and polymorphism of the gene encoding vascular endothelial growth factor (VEGF), which regulates vasculogenesis and angiogenesis [34, 35], mutation of the gene encoding Neuropilin1 (NRP1), a membrane co-receptor of VEGF [36], and mutation of the gene encoding Jagged1 (JAG1, also designated as CD339), that is, a cell surface protein interacting with receptors in the mammalian Notch signaling pathway [37], and also trisomy of chromosome 21, that is, Down's syndrome [33].

3. Vascular smooth muscle cells in healthy adult vessels

In healthy adult blood vessels, the vast majority of VSMCs are in a quiescent non-proliferative phenotype. This phenotype is usually defined as contractile, while the phenotype of proliferating VSMCs has been widely referred to as synthetic. However, the population of quiescent nonproliferating VSMCs is highly heterogeneous, varying dynamically from primarily contractile cells to synthetic cells specialized in extracellular matrix production [38]. In addition, the blood vessel wall contains abundant stem/progenitor cells, which are largely responsible for VSMC accumulation in the intima during vascular remodeling, such as neointimal hyperplasia and arteriosclerosis [10, 39–41]).

The degree of VSMC differentiation can be determined by the level of expression of specific markers of VSMC differentiation at mRNA and protein level. Markers of VSMC differentiation have been divided into early, mid-term and late, according their appearance during embryonic development [42], during restoration of differentiated phenotype of VSMC [7] or during differentiation of stem cells toward VSMCs [43, 44]. For example, during the early stage of differentiation of embryonic stem cell-derived embryoid bodies, SM α-actin is the first to be detected, followed by myocardin, SM22-α and smooth muscle myosin heavy chain (SM-MHC). The expression of SM-α actin, myocardin, SM22-α and SM-MHC was found to begin on day 0, 8, 11, 13, respectively, during early embryonic vascular development [42].

Early markers of VSMC differentiation include SM α-actin, myocardin and SM22-α [7, 43, 45]. Mid-term markers are h-caldesmon and SM-calponin [46–50], although these markers have been designated in other studies as early markers [44]. Late markers are desmin, meta-vinculin, SM-1 and SM-2 isoforms of myosin heavy chain, and smoothelin [7, 43, 44]. Markers of VSMC differentiation, stained by immunofluorescence, are demonstrated in **Figure 5** [8, 51–57].

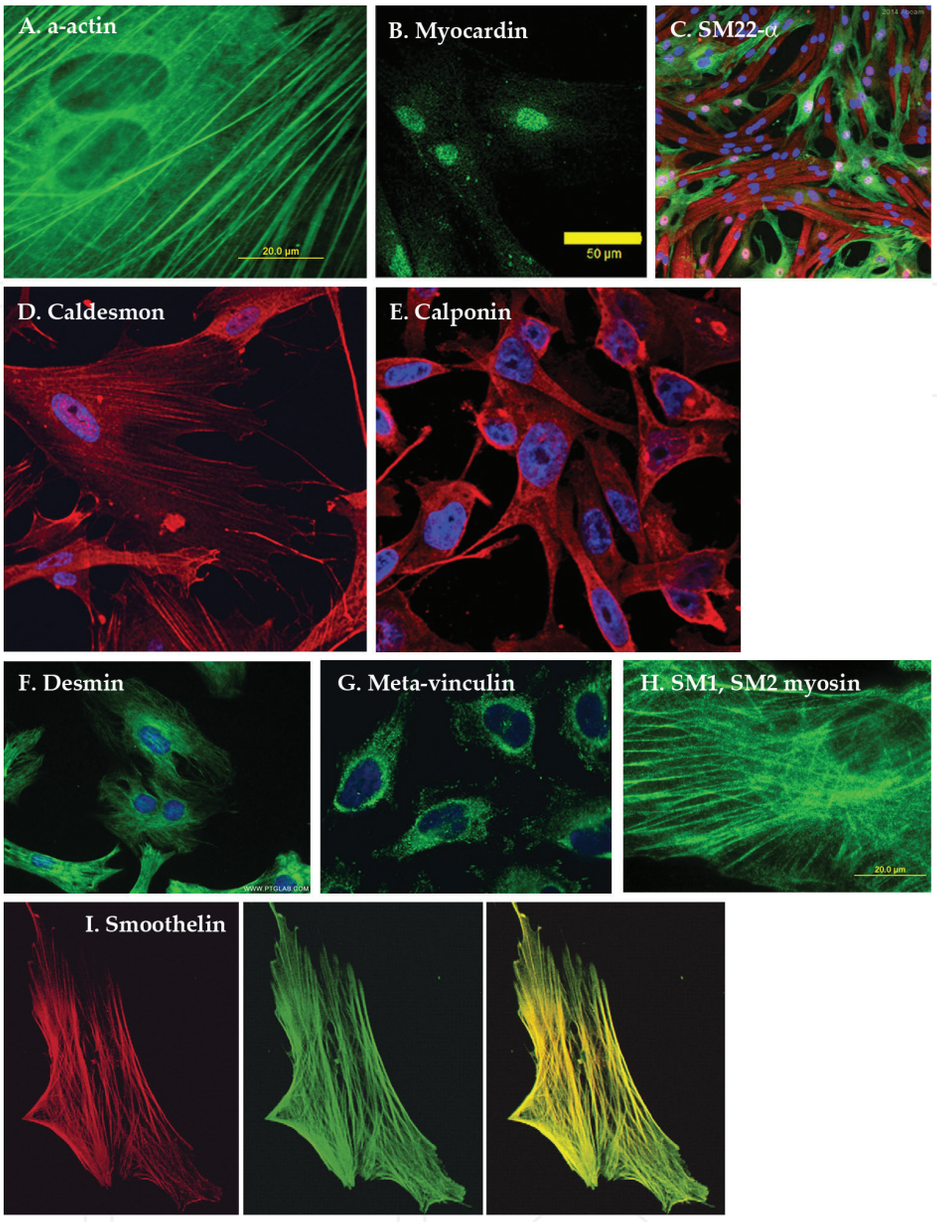

Figure 5. Markers of early (**A–C**), intermediate (**D, E**) and late (**F–I**) differentiation of VSMCs. **A–C, F–H**: Green fluorescence; **D, E, I**: Red fluorescence. I: Co-localization of smoothelin with alpha-actin: Left: Smoothelin, center: α-actin, right: Merge. A: [51], B: [52], C: [53], D: [54], E: [55], F: [56], G [57], H: [51], I: [8].

SM α-actin is the earliest known marker of VSMC differentiation, but its expression alone does not provide definitive evidence for a smooth muscle lineage [43]. SM α-actin is located in thin filaments of VSMCs, together with tropomyosin, troponin and calponin. Alpha-actin is the predominant isoform in the VSMCs of a healthy adult vessel. Other actin isoforms in VSMCs include β- and γ-actin, but they are minority components in adult physiological VSMCs, being localized predominantly in immature VSMCs during development or in dedifferentiated VSMCs in diseased vessels [4, 5, 9, 58].

Myocardin is a transcription factor essential for VSMC-specific differentiation. It is a transcriptional coactivator of the serum response factor involved in cell cycle regulation, apoptosis, cell growth and cell differentiation. Myocardin induces the expression of SM α-actin,

SM22-α, calponin and SM-MHC. Mice lacking myocardin die during embryogenesis from a lack of differentiated VSMCs. During supraphysiological mechanical load, for example during hypertension and prolonged stretching of VSMCs *in vitro*, myocardin is translocated from the nuclei to the cytoplasm and is degraded by the proteasome, which led to phenotypic modulation of VSMCs toward the synthetic and proliferative phenotype [9, 59–62].

SM22-α is an actin-binding protein of the calponin family that is involved in calcium-independent smooth muscle contraction [45, 63]. SM22-α interacts directly and co-localizes with F-actin, and it therefore participates in the organization of the actin cytoskeleton in differentiated VSMCs. SM22-α facilitates the assembly of actin filaments into bundles, enhances the contractility and the mobility of VSMCs, and maintains the contractile phenotype in VSMCs [64]. Disruption of SM22-α is involved in osteochondrogenesis in arterial diseases and also in vascular inflammation [45, 65]. SM22-α attenuated vascular inflammation by suppressing the IKK-IκBα-NF-κB signaling cascades [65].

Caldesmon is a cytoskeletal protein interacting with actin, tropomyosin, myosin, calmodulin and phospholipids. Due to alternative splicing of one gene, calmodulin occurs in two isoforms, namely high molecular weight (89–93 kDa) caldesmon isoforms (h-caldesmon), and low molecular weight (59–63 kDa) caldesmon isoforms (l-caldesmon). H-caldesmon is present in adult and fully differentiated smooth muscle cells, while l-caldesmon is found in non-muscle cells and in de-differentiated smooth muscle cells. H-caldesmon is also a marker of tumors, for example of soft tissue tumors of the skin. All isoforms are potent inhibitors of the actin-tropomyosin activated myosin MgATPase. Smooth muscle caldesmon, together with tropomyosin, is a mediating factor for Ca^{2+}-dependent inhibition of smooth muscle contraction [66, 67].

Calponin is another actin filament-associated regulatory protein expressed in smooth muscle cells and in many types of nonmuscle cells. It occurs in three isoforms, that is, calponin 1, 2 and 3, encoded with three homologous genes, CNN1, CNN2 and CNN3, respectively. All three isoforms inhibit actin-activated myosin ATPase and stabilize the actin cytoskeleton. Calponin 1 is specifically expressed in smooth muscle cells and plays a role in fine-tuning smooth muscle contractility. Similarly as in caldesmon, the interaction of calponin with actin inhibits actomyosin MgATPase activity. Calponin 2 is expressed both in smooth muscle cells and in nonmuscle cells, and it regulates multiple actin cytoskeleton-based functions. Calponin 3 participates in actin cytoskeleton-based activities in embryonic development, myogenesis and neuronal plasticity [68–70]. Another important role of calponin is its tumor-suppressing effect. The levels of calponin 1 and 2 have been found to be decreased in tumor cells, and transfection of these cells with gene encoding calponin 1 reduced their growth and malignancy. The level of calponin 2 in the serum of patients can also be used as a biomarker of tumor diseases, for example, breast cancer [70, 71].

Desmin, together with vimentin, forms intermediate filaments in VSMCs. During the development and specialization of cells toward smooth muscle cells, desmin replaces vimentin as the predominant component of intermediate filaments. Desmin is upregulated during differentiation of VSMCs from stem cells, for example, human bone marrow-derived mesenchymal stem cells (MSCs) [72] and also from embryonic mesothelial cells *via* epithelial-to-mesenchymal transition [14]. Desmin and smooth muscle myosin were expressed together in the cells, and their acquisition appeared indicative of the terminal differentiation of smooth muscle [73]. However, the amount of desmin was found to be much lower in VSMCs than in smooth muscle cells of the digestive, respiratory, and urogenital tract, and also much lower

than the amount of vimentin in VSMCs [58]. For example, rat aortic smooth muscle cells contain 51% of vimentin alone-positive cells, 48% with both vimentin and desmin and 1% with desmin alone [4].

Meta-vinculin is a high-molecular form of vinculin, that is, a protein of the focal adhesion plaques associated with integrin adhesion receptors in cells [74]. Meta-vinculin and vinculin are co-localized in focal adhesion plaques [75]. Together with SM-MHC, meta-vinculin is considered as a marker of well-differentiated contractile VSMCs [76, 77]). During phenotypic modulation of VSMCs, for example, in venous grafts used as aortocoronary bypasses [75], in human coronary arteries affected by arteriosclerosis [78] or during cultivation of VSMCs [75], the content of meta-vinculin in VSMCs decreases, and meta-vinculin can fully disappear from cells [75]. In cell culture conditions, the content of meta-vinculin in VSMCs can be reestablished when the cells reach confluence [79]. During hypoxic pulmonary hypertension induced in newborn calves, proliferation occurred almost exclusively in the meta-vinculin-negative VSMC population rather than in the VSMC population expressing meta-vinculin [80].

SM-1 and SM-2 isoforms of myosin heavy chain (SM-MHC) are located in thick filaments of fully differentiated VSMCs. In intact adult rat thoracic aorta, the ratio of SM-1:SM-2 is 80:20 both for mRNA and for protein [7]. VSMCs also contain the non-muscle (NM) isoform of myosin heavy chain, which is present, for example, in fibroblasts, macrophages, lymphocytes and platelets [81, 82]. The expression of myosin heavy chain isoforms in VSMCs is highly variable. The same VSMC cell can contain all three isoforms of myosin heavy chain or only SM myosins [7, 83]. This leads to a functional diversity of VSMCs, which have a fast contractile gene program, giving rise to a phasic smooth muscle phenotype, or a slow contractile gene program, giving rise to a tonic smooth muscle phenotype [84]. During vascular diseases, such as atherosclerosis [74], after vascular surgery, for example, coronary angioplasty [85] or after explantation of VSMCs *in vitro* [7], the expression of SM-1 myosin, and particularly of SM-2 myosin, decreases rapidly, while the content of NM myosin increases.

Smoothelin (SMTN) is a cytoskeletal protein present exclusively in contractile smooth muscle cells. The SMTN family of proteins consists of two isoforms of SMTN, namely SMTN-A, SMTN-B, and the SMTN-like protein 1 (SMTNL1). The SMTN-A isoform is located predominantly in visceral smooth muscle cells, while the SMTN-B isoform is located in vascular smooth muscle cells, and SMTNL1 is located in both visceral and vascular smooth muscle cells. SMTN-A and SMTNL1 are associated with the contractile apparatus in VSMCs, and their main function is to increase the contraction potential of VSMCs, to contribute to vascular adaptations in various physiological and pathological conditions, such as pregnancy, exercise training or hypertension and to contribute to the maintenance of the contractile phenotype [86, 87].

4. Vascular smooth muscle cells in vascular pathology

Typical examples of vascular diseases accompanied by vascular remodeling are atherosclerosis and hypertension. These two diseases have several common features, mainly thickening of the vessel wall and narrowing of the vascular lumen, which is due, among other reasons, to the activation of migration, growth and proteosynthesis in VSMCs.

4.1. Atherosclerosis

The onset of atherosclerosis is usually associated with mechanical or biochemical damage to the endothelial cell layer. Mechanical damage occurs during hypertension or during vascular surgery. Biochemical damage is due to the presence of various harmful compounds in the blood, for example, reactive oxygen species (ROS), nicotine or products of nonenzymatic glycation, and also due to elevated concentrations of originally physiological biomolecules, such as glucose, cholesterol or homocysteine. Biochemically damaged endothelium becomes thrombogenic, permeable and immunogenic. Platelets adhering to the endothelium then release platelet-derived growth factor (PDGF), a potent mitogen and chemoattractant for VSMCs [88, 89]. The production of PDGF-like molecules and other growth factors, for example VEGF, is also activated in the VSMCs themselves [90]. Other examples of growth factors that can penetrate into the vascular wall from the blood through the damaged endothelial barrier, and that can stimulate the proliferation of VSMCs, include epidermal growth factor (EGF), fibroblast growth factor (FGF) [91], nerve growth factor (NGF) [92], insulin-like growth factor-1 (IGF-1) [93] and TGF–β [11]. In addition to growth factors, other blood-borne molecules that can penetrate into the blood vessel wall can change the composition of its extracellular matrix (ECM) and can influence the behavior of VSMCs, include albumin, globulins including immunoglobulins, hemoglobin, lipoproteins, fibrinogen, fibrin, thrombin and thrombospondin [94–98].

Damaged endothelium is also prone to inflammatory activation. Endothelial cells increase their expression of adhesion molecules of immunoglobulin and selectin families, namely intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and endothelial-leucocyte adhesion molecule-1 (ELAM-1) [6, 11]. These molecules, present on the membrane of endothelial cells, bind the cells of the immune system, namely leucocytes, lymphocytes, monocytes, macrophages and mast cells. These cells are then stimulated by chemotactic factors to migrate inside the vascular wall. The chemotactic factors include interleukins (e.g. interleukin-1), tumor necrosis factors (TNFs, e.g. TNF-α) and monocyte chemoattractant protein-1 (MCP-1), and are produced in VSMCs, endothelial cells and inflammatory cells [11, 99]. In addition, immunoglobulin adhesion molecules, such as VCAM-1, can be expressed directly by VSMCs [100]. Another potent source of inflammatory cells penetrating inside the vascular wall is *tunica adventitia*, particularly its *vasa vasorum* [40, 101]. As concerns infiltration of the vascular wall with inflammatory cells, atherosclerosis has been considered as a specific type of inflammation [4, 102, 103]. In addition, the inflammatory cells produce proteolytic enzymes, such as chymase, tryptase and metalloproteases (MMPs, e.g. MMP-2, MMP-9 and MMP-13), which degrade the ECM and liberate the VSMCs from their proliferation control exerted by ECM [11, 104, 105]. The inflammatory cells also produce ROS, which can cause cell death in high concentrations. In low concentrations, however, they can stimulate phenotypic modulation and proliferation of VSMCs directly or indirectly by damage to the vascular ECM [11, 106]. Macrophages, like VSMCs, can proliferate within the damaged vascular wall [4, 107] and can also store lipids and form so-called foam cells (**Figure 6**) [11].

Under all these pathological conditions, VSMCs in the damaged vascular wall undergo phenotypic modulation, migrate to the *tunica intima* and proliferate. The proliferation of VSMCs can be very massive and can lead to considerable stenosis or to full obliteration of the vascular lumen. For this reason, early investigators of atherogenesis compared the proliferation

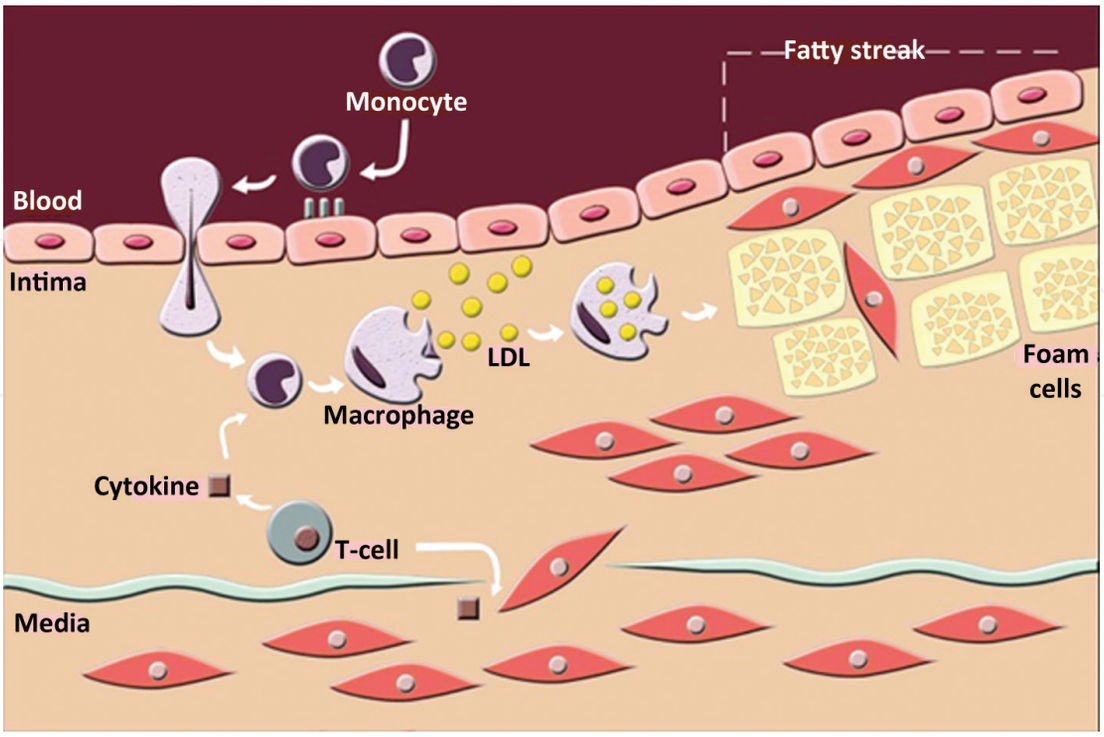

Figure 6. A scheme of the process of atherogenesis [11].

of VSMCs to the growth of tumor cells and considered atherosclerotic plaques as "benign tumors of VSMCs" [108, 109].

During phenotypic modulation, the late markers of VSMC differentiation are the first to be lost, while the early markers can persist even in migrating and proliferating cells. For example, smoothelin-B is the first smooth muscle cell marker that disappears when vascular tissues are compromised, for example, in atherosclerosis or restenosis [8]. Smoothelin was not detected in primary or long-term smooth muscle cell cultures, which simulate the pathological conditions in damaged blood vessels *in vivo* [86]. Similarly, in cultured VSMCs derived from the rat thoracic aorta and grown in 10% serum for 3–5 days to sub-confluence, the expression of SM-1 myosin at mRNA level decreased by 30% and the expression of SM-2 myosin at mRNA level decreased by 80%. At the protein level, SM-1 myosin was detectable at a reduced level in confluent cells, whereas SM-2 myosin was absent in confluent cells. Cultivation of confluent cells in serum-free media, that is under conditions used for restoration of the contractile phenotype, had little or no effect on SM-1 or SM-2 myosins at the mRNA level. In contrast, the level of SM α-actin, the earliest marker of VSMC differentiation, decreased both at the mRNA level and at the protein level. However, it did not fully disappear in these cells, and growth arrest by serum withdrawal or by high cell population density led to renewed SM α-actin expression in these cells [7]. In addition, phenotypically modulated VSMCs synthesize a spectrum of ECM molecules, which is altered quantitatively and qualitatively. For example, these VSMCs synthesize higher amounts of collagen type I, III and V, elastin and glycosaminoglycan, particularly chondroitin sulfate A/C and dermatan sulfate [6, 110]. In addition, phenotypically-modulated VSMCs synthesize ECM molecules, which are typical for osteoblasts and are involved in matrix calcification, for example, osteopontin and osteonectin [111, 112]. These VSMCs also showed increased expression and

DNA-binding activity of a transcription factor named Core binding factor alpha1 (Cbfa1) [111], expression of Runt-related transcription factor-2 (Runx2) [113], and increased expression and activity of alkaline phosphatase, an enzyme involved in matrix mineralization [111].

4.2. Hypertension

Two types of hypertension should be distinguished, namely **systemic hypertension** and **pulmonary hypertension**. Systemic hypertension can be a cause or a consequence of atherosclerosis, while pulmonary hypertension does not depend on the systemic blood pressure and has specific causes of its own, mainly pulmonary hypoxia.

Both types of hypertension include phenotypic modulation and proliferation of VSMCs, that is hyperplasia, which means an increasing number of VSMCs. In addition, **systemic hypertension** is characterized by the hypertrophy of VSMCs, that is an increase in VSMC volume. This hypertrophy is often associated with polyploidy, mainly tetraploidy and, to a lesser extent, also octoploidy. The polyploidization of VSMCs has been attributed to so-called "incomplete growth stimulation," which is sufficient only for DNA synthesis and the onset of mitosis, but not for subsequent karyokinesis and cytokinesis. Incomplete growth stimulation has been attributed to an increased level of contractile agonists, such as angiotensin II, arginine vasopressin (also known as antidiuretic hormone), adrenaline, bradykinin and serotonin, which act as weak mitogens for VSMCs [114, 115]). Incomplete mitosis without karyokinesis and cytokinesis is referred to as endomitosis [116]. If only cytokinesis is absent, binucleated or multinucleated, VSMCs are formed. If only DNA synthesis occurs, without complete mitosis, the process of cell polyploidization is referred to as endoreduplication. The increase in volume and ploidy in VSMCs has often been referred to as "specific hypertrophy," and has been considered by some investigators as a physiological response of VSMCs to mechanical loading during blood circulation, that is, as a certain type of VSMC differentiation. Polyploid VSMCs are more effective in synthesis of contractile proteins and mechanically resistant ECM proteins, and occur even under physiological conditions. For example, the aorta of healthy young rats contains 8–10% of polyploid VSMCs. However, during hypertension and with increasing age, the number of polyploid VSMCs can reach several tens % [114, 116, 117]. For this reason, the polyploidy of VSMCs has been proposed as a biomarker of senescence [118]. In addition, contractile proteins, such as actin and myosin, are not synthesized in proper isoforms typical for differentiated VSMCs. In other words, polyploidy of VSMCs appears to be associated with decreased-to-absent expression of muscle-specific proteins [119]. Accordingly, the maximal force per cross-sectional area generated by the hypertrophic smooth muscle in aorta from hypertensive rats was lower than in normal rat aorta [120]. In addition, increased synthesis of ECM proteins (e.g., collagen III, fibronectin) can lead to increased stiffness of the blood vessel wall, which further worsens the hypertension [115].

The main cause of **pulmonary hypertension** is alveolar hypoxia, which is due to a lower concentration of oxygen in the atmosphere, for example, at high altitudes [121] or in experimental isobaric or hypobaric hypoxic chambers [122–124]. Alveolar hypoxia also occurs during obstruction of airways, for example, during bronchial asthma [125–126] and chronic obstructive pulmonary disease (including pulmonary emphysema) [121, 127, 128], during interstitial fibrosis, which hampers the diffusion of oxygen from the alveoles to the capillaries [121, 128], during sterile and microbial inflammations in lungs [124, 126], during thromboembolism in pulmonary arteries [129] and also during extrapulmonary diseases, such as liver diseases [130] and cardiac diseases [131]).

http://dx.doi.org/10.5772/intechopen.77115

Pulmonary hypertension leads to phenotypic modulation and hyperplasia of VSMCs [121, 132, 133], but usually not to VSMC polyploidization. Surprisingly, the number of tetraploid VSMCs in pulmonary arteries even decreased, as revealed by flow cytometry of pulmonary arterial medial cells obtained from calves exposed to hypoxia in a hypobaric hypoxic chamber [122]. Tetraploid VSMCs were found in the pulmonary arteries of Eker rats, that is an animal model of somatic mutations in the tuberous sclerosis complex-2 (TSC2) gene [134].

Other factors associated with pulmonary hypertension include vasoconstriction [121, 131], damage to VSMCs by ROS [121], synthesis of ECM [132], degradation of ECM proteins by proteases [104, 105, 123], infiltration of the vascular wall with immunocompetent cells, particularly mast cells [104, 105], and inflammatory activation of VSMCs [132, 133, 135]. In addition, not only VSMCs in the *tunica media*, but also fibroblasts in the *tunica adventitia* migrate, proliferate and undergo inflammatory activation, and they therefore contribute to vascular remodeling [133, 136].

4.3. Other factors influencing the phenotype and proliferation of VSMCs

The propensity of VSMCs to phenotypic modulation and activation of migration and proliferation can also be influenced by their origin, their location in the vascular system, species, strain, breeding conditions, age and gender.

As mentioned earlier, VSMCs are of **neuroectodermal and mesodermal origin**. These two types of VSMCs respond in a different manner to various factors playing roles in the pathogenesis of vascular diseases acquired in adulthood, for example, atherosclerosis. For example, the *tunica media* of the aortic arch composed of VSMCs of neural crest origin calcified significantly earlier than the *tunica media* of the descending aorta composed of mesoderm-derived VSMCs [137]. Fluid shear stress, another factor contributing to the development of vascular diseases, inhibited the proliferation of mesodermal VSMCs but induced the proliferation of neuroectodermal VSMCs by increasing the expression of cyclin D1 (which mediates cell cycle progression from the G1 phase to the DNA-replicative S phase), by downregulating the cell cycle inhibitor p21 and by activating the Akt pathway in a manner dependent on phosphoinositide 3-kinase [138]. In addition, mesodermal VSMCs derived from avian embryonic vessels expressed about 10 times more SM α-actin and tropoelastin than neuroectodermal VSMCs [139].

An example of **regional differences** in VSMC growth is the higher incidence of polyploid VSMCs in the aorta and in other big elastic arteries than in smaller muscular arteries, for example, mesenteric arteries [114, 115]. A possible explanation is the lower mechanical load of big arteries, leading to relatively little damage to the endothelial barrier and lower permeability of this barrier for mitogens from blood. Another explanation is that elastin keeps the VSMCs in a contractile quiescent state, and these VSMCs are therefore less responsive to growth stimulation and undergo incomplete mitosis [140, 141]. Other examples include higher resistance of human VSMCs from the internal mammary artery to dedifferentiation and induction of migration and proliferation in comparison with VSMCs from other arteries, particularly VSMCs from coronary arteries [142], and a higher propensity of human arterial VSMCs than of venous VSMCs to form atherosclerotic lesions [143]. In spite of this, phenotypic alterations to venous VSMCs appear to be critical for the development of primary varicose veins. The VSMCs of varicose veins showed a lower expression of desmuslin, an intermediate filament protein, which

resulted in decreased expression of SM α-actin, SM-MHC and smoothelin, disassembly of actin stress fibers and increased levels of collagen synthesis and MMP-2 expression [144]. Phenotypic modulation, migration and proliferation of VSMC intima also occurs in vein grafts implanted in arterial position, for example, as an aortocoronary bypass. The VSMCs in vein grafts decreased their expression of myocardin, SM-1 and SM-2 myosins [61] and meta-vinculin [74]. Phenotypic modulation and proliferation of VSMCs in vein grafts can be attenuated by a perivascular drug delivery system releasing sirolimus and preventing distension of the vein grafts [145, 146], and also by transduction of VSMCs with microRNA-145-encoding plasmids [61].

However, it should be pointed out that, in addition to regional differences in the VSMC phenotype and growth, the VSMC population of **the same vessel** is highly heterogeneous, containing a wide spectrum of VSMCs varying from primarily contractile phenotype to synthetic cells specialized in extracellular matrix production, and also less-differentiated progenitor cells [10, 38–41].

Species-specific differences have been found, for example, in the expression of isoforms of arginase, that is an enzyme that stimulates VSMC proliferation and collagen deposition, and thus implicated in the vascular damage during atherosclerosis and during systemic and pulmonary hypertension. Specifically, rat VSMCs expressed isoform I of arginase, while human VSMCs expressed only arginase II [147]. Another example is a species-specific difference in VSMC-endothelial cell interaction. In co-culture with endothelial cells, the proliferation of VSMCs derived from human aorta was inhibited, while the proliferation of bovine aortic VSMCs was stimulated [148].

Strain-specific differences in VSMC growth have been studied mainly in rats. It was found that polyploidization of aortic VSMCs was the highest in the Wistar-Kyoto (WKY) strain of rats (i.e., normotensive inbred rats related to spontaneously hypertensive rats, SHR); it was intermediate in SHR (genetically hypertensive rat), and it was lowest in Sprague-Dawley rats (i.e., normotensive outbred rats) and in Fischer rats (i.e., normotensive inbred rats). Nonarterial cells (venous VSMCs and lung cells) from WKY and SHR remained essentially diploid, suggesting that polyploidization is also tissue-specific [149]. At the same time, the proliferation, that is hyperplasia, of VSMCs derived from the aorta of SHR rats was markedly higher than that found in aortic VSMCs from WKY rats [150]. The propensity of VSMCs to migration and proliferation can also be influenced by **breeding conditions**. In our earlier study, the migration and proliferation of cultured aortic VSMC derived from Wistar rats raised under conventional conditions was higher than in cells from Wistar rats raised under specific pathogen-free (SPF) conditions [151].

The phenotype and growth of VSMCs can also be influenced by the **age of the organism**. The proliferation activity of fetal and neonatal VSMCs is higher, and their differentiation status is physiologically lower, than in adult VSMCs [152, 153]. In adult organisms, increased age is associated mainly with negative factors affecting VSMCs, for example, oxidative damage, DNA damage (including telomere attrition), mitochondrial dysfunction, apoptosis, pro-inflammatory secretory phenotype associated with the loss of VSMC differentiation markers, such as SM α-actin and SM22-α, increased expression of transcription factors Msx2 and Runx2 and of bone morphogenetic protein-2, that is markers of osteoblast transition of VSMCs, and increased sensitivity of β-adrenoceptors, which are implicated in the inhibition of cellular

proliferation [154–157]. These changes in VSMC behavior caused by donor age can be further magnified by proliferative aging of VSMCs during their cultivation *in vitro* [156].

Gender plays an important role in the propensity of VSMCs to migration and proliferation. It is generally known that estrogens decrease the proliferative activity of VSMCs, while androgens increase it. This is considered as the main cause of the higher incidence of cardiovascular diseases in male organisms. However, VSMCs from males proliferated faster even without the actual presence of sex hormones in the cell culture medium. For example, VSMCs isolated from the thoracic aorta of adult male rats migrated earlier from the explants and proliferated faster than their female counterparts [158, 159]. These differences were enhanced in a serum-free medium [160] and after repeated passaging of VSMCs [161], and were also apparent in newborn rats [162] and in both WKY rats and SHR rats [150]. These differences have been explained by prenatal synthesis of androgens initiated by the expression of specific genes in the SRY locus on the Y chromosome. Among others, androgens increase the sensitivity of VSMCs to adrenergic hormones, which persists throughout life, and also without the actual presence of physiological levels of androgens [163, 164].

5. Conclusion

Vascular smooth muscle cells (VSMCs) are physiological and the most numerous component of the arterial and venous wall, and they ensure vasoconstriction and vasodilatation and other functions, such as synthesis of extracellular matrix. However, VSMCs are also implicated in vascular disorders, such as defects of cardiovascular morphogenesis, atherosclerosis, and systemic and pulmonary hypertension. The VSMCs in healthy adult blood vessels are in the quiescent contractile state, characterized by specific markers of VSMC differentiation namely SM α-actin, myocardin and SM22-α (early markers), h-caldesmon and SM calponin (intermediate markers) and desmin, meta-vinculin, SM-1 and SM-2 isoforms of myosin heavy chain and smoothelin (late markers). However, in pathologically changed blood vessels, VSMCs lose their differentiation markers, activate migration and proliferation and increase proteosynthesis. This VSMC behavior can lead to remodeling of the vascular wall, including stenosis and obliteration of the vascular lumen.

Acknowledgements

This study was supported by the Czech Health Research Council, Ministry of Health of the Czech Republic (project No. 15-33018A). Further support was provided by BIOCEV – Biotechnology and Biomedicine Centre of the Academy of Sciences and Charles University (CZ.1.05/1.1.00.02.0109), a European Regional Development Fund project. Mr. Robin Healey (Czech Technical University in Prague) is gratefully acknowledged for his language revision of the manuscript.

Conflict of interest

The authors declare no conflict of interest.

Author details

Lucie Bacakova[1,2]*, Martina Travnickova[1,2], Elena Filova[1,2], Roman Matějka[1,2], Jana Stepanovska[1,2], Jana Musilkova[1], Jana Zarubova[1,2] and Martin Molitor[3]

*Address all correspondence to: lucie.bacakova@fgu.cas.cz

1 Department of Biomaterials and Tissue Engineering, Institute of Physiology of the Czech Academy of Sciences, Videnska, Prague, Czech Republic

2 Biotechnology and Biomedicine Center of the Academy of Sciences and Charles University in Vestec (BIOCEV), Prumyslova, Vestec, Czech Republic

3 Clinic of Plastic Surgery, Faculty Hospital Na Bulovce, Budinova, Prague, Czech Republic

References

[1] Presentation of Abdelaty Shawky, Assoc. Prof. of Pathology. Available from: http://slideplayer.com/slide/8073072/ [Accessed: 2017-02-19]

[2] Man Anatomy. Veins. Available from: https://www.mananatomy.com/basic-anatomy/veins. [Accessed: 2017-02-19]

[3] Exam 1 Review: Chapter 19: Capillaries. Available from: http://www.apsubiology.org/anatomy/2020/2020_Exam_Reviews/Exam_1/CH19_Capillaries.htm. [Accessed: 2017-02-19]

[4] Schwartz SM, Campbell GR, Campbell JH. Replication of smooth muscle cells in vascular disease. Circulation Research. 1986;**58**(4):427-444. DOI: 10.1161/01.RES.58.4.427

[5] Campbell GR, Campbell JH. The phenotypes of smooth muscle expressed in human atheroma. Annals of the New York Academy of Sciences. 1990;**598**:143-158. DOI: 10.1111/j.1749-6632.1990.tb42286.x

[6] Campbell JH, Campbell GR. Smooth muscle phenotypic modulation--a personal experience. Arteriosclerosis, Thrombosis, and Vascular Biology. 2012;**32**(8):1784-1789. DOI: 10.1161/ATVBAHA.111.243212

[7] Babij P, Kawamoto S, White S, Adelstein RS, Periasamy M. Differential expression of SM1 and SM2 myosin isoforms in cultured vascular smooth muscle. The American Journal of Physiology. 1992;**262**(3 Pt 1):C607-C613. DOI: 10.1152/ajpcell.1992.262.3.C607

[8] van Eys GJ, Niessen PM, Rensen SS. Smoothelin in vascular smooth muscle cells. Trends in Cardiovascular Medicine. 2007;**17**(1):26-30. DOI: 10.1016/j.tcm.2006.11.001

[9] Shinohara S, Shinohara S, Kihara T, Miyake J. Regulation of differentiated phenotypes of vascular smooth muscle cells, Chapter 14. In: Sugi H, editor. Current Basic and Pathological Approaches to the Function of Muscle Cells and Tissues - from Molecules to Humans. InTech; 2012. pp. 331-344. DOI: 10.5772/3003, ISBN 978-953-51-0679-1. Available from: https://www.intechopen.com/books/current-basic-and-pathological-

approaches-to-the-function-of-muscle-cells-and-tissues-from-molecules-to-humans/regulation-of-differentiated-phenotypes-of-vascular-smooth-muscle-cells

[10] Wang G, Jacquet L, Karamariti E, Xu Q. Origin and differentiation of vascular smooth muscle cells. The Journal of Physiology. 2015;**593**(14):3013-3030. DOI: 10.1113/JP270033

[11] Saleh Al-Shehabi T, Iratni R, Eid AH. Anti-atherosclerotic plants which modulate the phenotype of vascular smooth muscle cells. Phytomedicine. 2016;**23**(11):1068-1081. DOI: 10.1016/j.phymed.2015.10.016

[12] Skalli O, Bloom WS, Ropraz P, Azzarone B, Gabbiani G. Cytoskeletal remodeling of rat aortic smooth muscle cells in vitro: Relationships to culture conditions and analogies to in vivo situations. Journal of Submicroscopic Cytology. 1986;**18**(3):481-493. PMID: 3528513

[13] Hungerford JE, Owens GK, Argraves WS, Little CD. Development of the aortic vessel wall as defined by vascular smooth muscle and extracellular matrix markers. Developmental Biology. 1996;**178**(2):375-392. DOI: 10.1006/dbio.1996.0225

[14] Lachaud CC, López-Beas J, Soria B, Hmadcha A. EGF-induced adipose tissue mesothelial cells undergo functional vascular smooth muscle differentiation. Cell Death & Disease. 2014;**5**:e1304. DOI: 10.1038/cddis.2014.271

[15] Etchevers HC, Vincent C, Le Douarin NM, Couly GF. The cephalic neural crest provides pericytes and smooth muscle cells to all blood vessels of the face and forebrain. Development. 2001;**128**(7):1059-1068. 11245571

[16] Sieber-Blum M. Cardiac neural crest stem cells. The Anatomical Record. Part A, Discoveries in Molecular, Cellular, and Evolutionary Biology. 2004;**276**(1):34-42. DOI: 10.1002/ar.a.10132

[17] Sawada H, Rateri DL, Moorleghen JJ, Majesky MW, Daugherty A. Smooth muscle cells derived from second heart field and cardiac neural crest reside in spatially distinct domains in the media of the ascending aorta - brief report. Arteriosclerosis, Thrombosis, and Vascular Biology. 2017;**37**(9):1722-1726. DOI: 10.1161/ATVBAHA.117.309599

[18] Brown CB, Baldwin HS. Control neural crest contribution to the cardiovascular system. Advances in Experimental Medicine and Biology. 2006;**589**:134-154. DOI: 10.1007/978-0-387-46954-6_8

[19] Le Lièvre CS, Le Douarin NM. Mesenchymal derivatives of the neural crest: Analysis of chimaeric quail and chick embryos. Journal of Embryology and Experimental Morphology. 1975;**34**(1):125-154. 1185098

[20] Bergwerff M, Verberne ME, DeRuiter MC, Poelmann RE, Gittenberger-de Groot AC. Neural crest cell contribution to the developing circulatory system: Implications for vascular morphology? Circulation Research. 1998;**82**(2):221-231. DOI: 10.1161/01.RES.82.2.221

[21] High FA, Zhang M, Proweller A, Tu L, Parmacek MS, Pear WS, Epstein JA. An essential role for notch in neural crest during cardiovascular development and smooth muscle differentiation. The Journal of Clinical Investigation. 2007;**117**(2):353-363. DOI: 10.1172/JCI30070

[22] Manderfield LJ, Aghajanian H, Engleka KA, Lim LY, Liu F, Jain R, Li L, Olson EN, Epstein JA. Hippo signaling is required for notch-dependent smooth muscle differentiation of neural crest. Development. 2015;**142**(17):2962-2971. DOI: 10.1242/dev.125807

[23] Wang X, Astrof S. Neural crest cell-autonomous roles of fibronectin in cardiovascular development. Development. 2016;**143**(1):88-100. DOI: 10.1242/dev.125286

[24] Li J, Zhu X, Chen M, Cheng L, Zhou D, Lu MM, Du K, Epstein JA, Parmacek MS. Myocardin-related transcription factor B is required in cardiac neural crest for smooth muscle differentiation and cardiovascular development. Proceedings of the National Academy of Sciences of the United States of America. 2005;**102**(25): 8916-8921. DOI: 10.1073/pnas.0503741102

[25] Xie WB, Li Z, Shi N, Guo X, Tang J, Ju W, Han J, Liu T, Bottinger EP, Chai Y, Jose PA, Chen SY. Smad2 and myocardin-related transcription factor B cooperatively regulate vascular smooth muscle differentiation from neural crest cells. Circulation Research. 2013;**113**(8):e76-e86. DOI: 10.1161/CIRCRESAHA.113.301921

[26] Topouzis S, Majesky MW. Smooth muscle lineage diversity in the chick embryo. Two types of aortic smooth muscle cell differ in growth and receptor-mediated transcriptional responses to transforming growth factor-beta. Developmental Biology. 1996;**178**(2): 430-445. DOI: 10.1006/dbio.1996.0229

[27] Jiao J, Xiong W, Wang L, Yang J, Qiu P, Hirai H, Shao L, Milewicz D, Chen YE, Yang B. Differentiation defect in neural crest-derived smooth muscle cells in patients with aortopathy associated with bicuspid aortic valves. eBioMedicine. 2016;**10**:282-290. DOI: 10.1016/j.ebiom.2016.06.045

[28] Sanchez-Castro M, Eldjouzi H, Charpentier E, Busson PF, Hauet Q, Lindenbaum P, Delasalle-Guyomarch B, Baudry A, Pichon O, Pascal C, Lefort B, Bajolle F, Pezard P, Schott JJ, Dina C, Redon R, Gournay V, Bonnet D, Le Caignec C. Search for rare copy-number variants in congenital heart defects identifies novel candidate genes and a potential role for FOXC1 in patients with coarctation of the aorta. Circulation. Cardiovascular Genetics. 2016;**9**(1):86-94. DOI: 10.1161/CIRCGENETICS.115.001213

[29] Skarie JM, Link BA. FoxC1 is essential for vascular basement membrane integrity and hyaloid vessel morphogenesis. Investigative Ophthalmology & Visual Science. 2009;**50**(11):5026-5034. DOI: 10.1167/iovs.09-3447

[30] Berry FB, Skarie JM, Mirzayans F, Fortin Y, Hudson TJ, Raymond V, Link BA, Walter MA. FOXC1 is required for cell viability and resistance to oxidative stress in the eye through the transcriptional regulation of FOXO1A. Human Molecular Genetics. 2008;**17**(4):490-505. DOI: 10.1093/hmg/ddm326

[31] Shin YW, Jung KH, Kim JM, Cho YD, Lee ST, Chu K, Kim M, Lee SK, Han MH, Roh JK. Echocardiographic evidence of innate aortopathy in the human intracranial aneurysm. PLoS One. 2014;**9**(6):e100569. DOI: 10.1371/journal.pone.0100569

[32] Yajima I, Colombo S, Puig I, Champeval D, Kumasaka M, Belloir E, Bonaventure J, Mark M, Yamamoto H, Taketo MM, Choquet P, Etchevers HC, Beermann F, Delmas V,

Monassier L, Larue L. A subpopulation of smooth muscle cells, derived from melanocyte-competent precursors, prevents patent ductus arteriosus. PLoS One. 2013;**8**(1):e53183. DOI: 10.1371/journal.pone.0053183

[33] Grunert M, Dorn C, Schueler M, Dunkel I, Schlesinger J, Mebus S, Alexi-Meskishvili V, Perrot A, Wassilew K, Timmermann B, Hetzer R, Berger F, Sperling SR. Rare and private variations in neural crest, apoptosis and sarcomere genes define the polygenic background of isolated tetralogy of Fallot. Human Molecular Genetics. 2014;**23**(12):3115-3128. DOI: 10.1093/hmg/ddu021

[34] Rammeloo LA, DeRuiter MC, van den Akker NM, Wisse LJ, Gittenberger-de Groot AC. Development of major aorto-pulmonary collateral arteries in vegf120/120 isoform mouse embryos with tetralogy of fallot. Pediatric Cardiology. 2015;**36**(1):89-95. DOI: 10.1007/s00246-014-0969-4

[35] Li X, Liu CL, Li XX, Li QC, Ma LM, Liu GL. VEGF gene polymorphisms are associated with risk of tetralogy of Fallot. Medical Science Monitor. 2015;**21**:3474-3482. DOI: 10.12659/MSM.894568

[36] Duran I, Tenney J, Warren CM, Sarukhanov A, Csukasi F, Skalansky M, Iruela-Arispe ML, Krakow D. NRP1 haploinsufficiency predisposes to the development of tetralogy of Fallot. American Journal of Medical Genetics. Part A. 2018;**176**(3):649-656. DOI: 10.1002/ajmg.a.38600

[37] Eldadah ZA, Hamosh A, Biery NJ, Montgomery RA, Duke M, Elkins R, Dietz HC. Familial tetralogy of Fallot caused by mutation in the jagged1 gene. Human Molecular Genetics. 2001;**10**(2):163-169. PMID: 11152664

[38] Drake CJ, Hungerford JE, Little CD. Morphogenesis of the first blood vessels. Annals of the New York Academy of Sciences. 1998;**857**:155-179. DOI: 10.1111/j.1749-6632.1998.tb10115

[39] Klein D, Hohn HP, Kleff V, Tilki D, Ergün S. Vascular wall-resident stem cells. Histology and Histopathology. 2010;**25**(5):681-689. DOI: 10.14670/HH-25.681

[40] Majesky MW, Dong XR, Hoglund V, Mahoney WM Jr, Daum G. The adventitia: A dynamic interface containing resident progenitor cells. Arteriosclerosis, Thrombosis, and Vascular Biology. 2011;**31**(7):1530-1539. DOI: 10.1161/ATVBAHA.110.221549

[41] Psaltis PJ, Harbuzariu A, Delacroix S, Holroyd EW, Simari RD. Resident vascular progenitor cells--diverse origins, phenotype, and function. Journal of Cardiovascular Translational Research. 2011;**4**(2):161-176. DOI: 10.1007/s12265-010-9248-9

[42] Han YL, Xiao YP, Qi YM, Kang J, Yan CH. Expression of vascular smooth muscle cell markers during early stage of embryonic stem cell-derived embryoid bodies differentiation. Zhongguo Ying Yong Sheng Li Xue Za Zhi. 2008;**24**(4):385-390. PMID: 21158132

[43] Rodríguez LV, Alfonso Z, Zhang R, Leung J, Wu B, Ignarro LJ. Clonogenic multipotent stem cells in human adipose tissue differentiate into functional smooth muscle cells. Proceedings of the National Academy of Sciences of the United States of America. 2006;**103**(32):12167-12172. DOI: 10.1073/pnas.0604850103

[44] Harris LJ, Abdollahi H, Zhang P, McIlhenny S, Tulenko TN, DiMuzio PJ. Differentiation of adult stem cells into smooth muscle for vascular tissue engineering. The Journal of Surgical Research. 2011;**168**(2):306-314. DOI: 10.1016/j.jss.2009.08.001

[45] Dong LH, Lv P, Han M. Roles of SM22α in cellular plasticity and vascular diseases. Cardiovascular & Hematological Disorders Drug Targets. 2012;**12**(2):119-125. DOI: 10.2174/1871529X11202020119

[46] Rashidi N, Tafazzoli-Shadpour M, Haghighipour N, Khani MM. Morphology and contractile gene expression of adipose-derived mesenchymal stem cells in response to short-term cyclic uniaxial strain and TGF-β1. Biomedizinische Technik. Biomedical Engineering. 2017, in press. pii: /j/bmte.ahead-of-print/bmt-2016-0228/bmt-2016-0228.xml. DOI: 10.1515/bmt-2016-0228

[47] Gong Z, Niklason LE. Small-diameter human vessel wall engineered from bone marrow-derived mesenchymal stem cells (hMSCs). The FASEB Journal. 2008;**22**(6):1635-1648. DOI: 10.1096/fj.07-087924

[48] Wang C, Yin S, Cen L, Liu Q, Liu W, Cao Y, Cui L. Differentiation of adipose-derived stem cells into contractile smooth muscle cells induced by transforming growth factor-beta1 and bone morphogenetic protein-4. Tissue Engineering. Part A. 2010;**16**(4):1201-1123. DOI: 10.1089/ten.TEA.2009.0303

[49] Xu ZC, Zhang Q, Li H. Human hair follicle stem cell differentiation into contractile smooth muscle cells is induced by transforming growth factor-β1 and platelet-derived growth factor BB. Molecular Medicine Reports. 2013;**8**(6):1715-1721. DOI: 10.3892/mmr.2013.1707

[50] Mesure B, Huber-Villaume S, Menu P, Velot É. Transforming growth factor-beta 1 or ascorbic acid are able to differentiate Wharton's jelly mesenchymal stem cells towards a smooth muscle phenotype. Bio-medical Materials and Engineering. 2017;**28**(s1):S101-S105. DOI: 10.3233/BME-171630

[51] Parizek M, Slepickova Kasalkova N, Bacakova L, Svindrych Z, Slepicka P, Bacakova M, Lisa V, Svorcik V. Adhesion, growth, and maturation of vascular smooth muscle cells on low-density polyethylene grafted with bioactive substances. BioMed Research International. 2013;**2013**:371430. DOI: 10.1155/2013/371430

[52] Hashemi Gheinani A, Burkhard FC, Rehrauer H, Aquino Fournier C, Monastyrskaya K. MicroRNA MiR-199a-5p regulates smooth muscle cell proliferation and morphology by targeting WNT2 signaling pathway. The Journal of Biological Chemistry. 2015;**290**(11):7067-7086. DOI: 10.1074/jbc.M114.618694

[53] Abcam. Anti-SM22 alpha antibody (ab14106). Available from: http://www.abcam.com/products?selected.researchAreas=Cardiovascular--Cardiovascular+Markers--Cell+Markers--Smooth+Muscle+Cells. [Accessed: 2017-02-19]

[54] R & D Systems. Human Caldesmon/CALD1 Antibody. Available from: https://www.rndsystems.com/products/human-caldesmon-cald1-antibody-767001_mab7569#ds_image_1 [Accessed: 2017-02-19]

[55] Human Calponin 1 Antibody. Available from: https://www.rndsystems.com/products/human-calponin-1-antibody-836701_mab7900. [Accessed: 2017-02-19]

[56] Proteintech. Desmin Antibody Rabbit Polyclonal, catalog number: 16520-1-AP. Available from: http://www.ptglab.com/products/DES-Antibody-16520-1-AP.htm. [Accessed: 2017-02-19]

[57] GeneTex. Vinculin antibody [N1N3]. Available from https://www.genetex.com/Vinculin-antibody-N1N3-GTX113294.html [Accessed: 2017-02-19]

[58] Gabbiani G, Schmid E, Winter S, Chaponnier C, de Ckhastonay C, Vandekerckhove J, Weber K, Franke WW. Vascular smooth muscle cells differ from other smooth muscle cells: Predominance of vimentin filaments and a specific alpha-type actin. Proceedings of the National Academy of Sciences of the United States of America. 1981;**78**(1):298-302. PMID: 7017714

[59] Kiyan Y, Limbourg A, Kiyan R, Tkachuk S, Limbourg FP, Ovsianikov A, Chichkov BN, Haller H, Dumler I. Urokinase receptor associates with myocardin to control vascular smooth muscle cells phenotype in vascular disease. Arteriosclerosis, Thrombosis, and Vascular Biology. 2012;**32**(1):110-122. DOI: 10.1161/ATVBAHA.111.234369

[60] Pfisterer L, Feldner A, Hecker M, Korff T. Hypertension impairs myocardin function: A novel mechanism facilitating arterial remodelling. Cardiovascular Research. 2012;**96**(1):120-129. DOI: 10.1093/cvr/cvs247

[61] Ohnaka M, Marui A, Yamahara K, Minakata K, Yamazaki K, Kumagai M, Masumoto H, Tanaka S, Ikeda T, Sakata R. Effect of microRNA-145 to prevent vein graft disease in rabbits by regulation of smooth muscle cell phenotype. The Journal of Thoracic and Cardiovascular Surgery. 2014;**148**(2):676-682.e2. DOI: 10.1016/j.jtcvs.2013.11.054

[62] Crosas-Molist E, Meirelles T, López-Luque J, Serra-Peinado C, Selva J, Caja L, Gorbenko Del Blanco D, Uriarte JJ, Bertran E, Mendizábal Y, Hernández V, García-Calero C, Busnadiego O, Condom E, Toral D, Castellà M, Forteza A, Navajas D, Sarri E, Rodríguez-Pascual F, Dietz HC, Fabregat I, Egea G. Vascular smooth muscle cell phenotypic changes in patients with Marfan syndrome. Arteriosclerosis, Thrombosis, and Vascular Biology. 2015;**35**(4):960-972. DOI: 10.1161/ATVBAHA.114.304412

[63] Je HD, Sohn UD. SM22alpha is required for agonist-induced regulation of contractility: Evidence from SM22alpha knockout mice. Molecules and Cells. 2007;**23**(2):175-181. PMID: 17464194

[64] Han M, Dong LH, Zheng B, Shi JH, Wen JK, Cheng Y. Smooth muscle 22 alpha maintains the differentiated phenotype of vascular smooth muscle cells by inducing filamentous actin bundling. Life Sciences. 2009;**84**(13-14):394-401. DOI: 10.1016/j.lfs.2008.11.017

[65] Shu YN, Zhang F, Bi W, Dong LH, Zhang DD, Chen R, Lv P, Xie XL, Lin YL, Xue ZY, Li H, Miao SB, Zhao LL, Wang H, Han M. SM22α inhibits vascular inflammation via stabilization of IκBα in vascular smooth muscle cells. Journal of Molecular and Cellular Cardiology. 2015;**84**:191-199. DOI: 10.1016/j.yjmcc.2015.04.020

[66] Huber PA. Caldesmon. The International Journal of Biochemistry & Cell Biology. 1997;**29**(8-9):1047-1051. DOI: 10.1016/S1357-2725(97)00004-6

[67] D'Addario SF, Morgan M, Talley L, Smoller BR. H-Caldesmon as a specific marker of smooth muscle cell differentiation in some soft tissue tumors of the skin. Journal of Cutaneous Pathology. 2002;**29**(7):426-429. DOI: 10.1034/j.1600-0560.2002.290707.x

[68] Winder SJ, Walsh MP. Calponin: Thin filament-linked regulation of smooth muscle contraction. Cellular Signalling. 1993;**5**(6):677-686. PMID: 8130072

[69] el-Mezgueldi M. Calponin. The International Journal of Biochemistry & Cell Biology. 1996;**28**(11):1185-1189. DOI: 10.1016/S1357-2725(96)00085-4

[70] Liu R, Jin JP. Calponin isoforms CNN1, CNN2 and CNN3: Regulators for actin cytoskeleton functions in smooth muscle and non-muscle cells. Gene. 2016;**585**(1):143-153. DOI: 10.1016/j.gene.2016.02.040

[71] Taniguchi S. Suppression of cancer phenotypes through a multifunctional actin-binding protein, calponin, that attacks cancer cells and simultaneously protects the host from invasion. Cancer Science. 2005;**96**(11):738-746. DOI: 10.1111/j.1349-7006.2005.00118.x

[72] Hegner B, Weber M, Dragun D, Schulze-Lohoff E. Differential regulation of smooth muscle markers in human bone marrow-derived mesenchymal stem cells. Journal of Hypertension. 2005;**23**(6):1191-1202. DOI: 10.1097/01.hjh.0000170382.31085.5d

[73] Mitchell JJ, Reynolds SE, Leslie KO, Low RB, Woodcock-Mitchell J. Smooth muscle cell markers in developing rat lung. American Journal of Respiratory Cell and Molecular Biology. 1990;**3**(6):515-523. DOI: 10.1165/ajrcmb/3.6.515

[74] Brinck U, Mirzaie M, Korabiowska M, Meyer T. Expression rate of vinculin isoforms in human aortocoronary saphenous vein grafts. International Journal of Cardiology. 1997;**59**(2):125-132. DOI: 10.1016/S0167-5273(97)02919-7

[75] Belkin AM, Ornatsky OI, Glukhova MA, Koteliansky VE. Immunolocalization of meta-vinculin in human smooth and cardiac muscles. The Journal of Cell Biology. 1988;**107**(2):545-553. DOI: 10.1083/jcb.107.2.545

[76] Glukhova MA, Kabakov AE, Belkin AM, Frid MG, Ornatsky OI, Zhidkova NI, Koteliansky VE. Meta-vinculin distribution in adult human tissues and cultured cells. FEBS Letters. 1986;**207**(1):139-141. DOI: 10.1016/0014-5793(86)80027-8

[77] Stiebellehner L, Frid MG, Reeves JT, Low RB, Gnanasekharan M, Stenmark KR. Bovine distal pulmonary arterial media is composed of a uniform population of well-differentiated smooth muscle cells with low proliferative capabilities. American Journal of Physiology. Lung Cellular and Molecular Physiology. 2003;**285**(4):L819-L828. DOI: 10.1152/ajplung.00062.2003

[78] Meyer T, Brink U, Unterberg C, Stöhr S, Kreuzer H, Buchwald AB. Expression of meta-vinculin in human coronary arteriosclerosis is related to the histological grade of plaque formation. Atherosclerosis. 1994;**111**(1):111-119. DOI: 10.1016/0021-9150(94)90196-1

[79] Shirinsky VP, Birukov KG, Koteliansky VE, Glukhova MA, Spanidis E, Rogers JD, Campbell JH, Campbell GR. Density-related expression of caldesmon and vinculin in cultured rabbit aortic smooth muscle cells. Experimental Cell Research. 1991;**194**(2):186-189. DOI: 10.1016/0014-4827(91)90352-U

[80] Wohrley JD, Frid MG, Moiseeva EP, Orton EC, Belknap JK, Stenmark KR. Hypoxia selectively induces proliferation in a specific subpopulation of smooth muscle cells in the bovine neonatal pulmonary arterial media. The Journal of Clinical Investigation. 1995;**96**(1):273-281. DOI: 10.1172/JCI118031

[81] Zanellato AM, Borrione AC, Tonello M, Scannapieco G, Pauletto P, Sartore S. Myosin isoform expression and smooth muscle cell heterogeneity in normal and atherosclerotic rabbit aorta. Arteriosclerosis. 1990;**10**(6):996-1009. DOI: 10.1161/01.ATV.10.6.996

[82] Sartore S, Chiavegato A, Franch R, Faggin E, Pauletto P. Myosin gene expression and cell phenotypes in vascular smooth muscle during development, in experimental models, and in vascular disease. Arteriosclerosis, Thrombosis, and Vascular Biology. 1997;**17**(7):1210-1215. DOI: 10.1161/01.ATV.17.7.1210

[83] Eddinger TJ, Meer DP. Myosin isoform heterogeneity in single smooth muscle cells. Comparative Biochemistry and Physiology. Part B, Biochemistry & Molecular Biology. 1997;**117**(1):29-38. PMID: 9180012

[84] Reho JJ, Zheng X, Fisher SA. Smooth muscle contractile diversity in the control of regional circulations. American Journal of Physiology. Heart and Circulatory Physiology. 2014;**306**(2):H163-H172. DOI: 10.1152/ajpheart.00493.2013

[85] Aikawa M, Sakomura Y, Ueda M, Kimura K, Manabe I, Ishiwata S, Komiyama N, Yamaguchi H, Yazaki Y, Nagai R. Redifferentiation of smooth muscle cells after coronary angioplasty determined via myosin heavy chain expression. Circulation. 1997;**96**(1): 82-90. DOI: 10.1161/01.CIR.96.1.82

[86] van der Loop FT, Schaart G, Timmer ED, Ramaekers FC, van Eys GJ. Smoothelin, a novel cytoskeletal protein specific for smooth muscle cells. The Journal of Cell Biology. 1996;**134**(2):401-411. DOI: 10.1097/PAI.0b013e31827387c9

[87] Turner SR, MacDonald JA. Novel contributions of the smoothelin-like 1 protein in vascular smooth muscle contraction and its potential involvement in myogenic tone. Microcirculation. 2014;**21**(3):249-258. DOI: 10.1111/micc.12108

[88] Ross R, Glomset JA, Kariya B, Harker L. A platelet-dependent serum factor that stimulates the proliferation of arterial smooth muscle cells in vitro. Proceedings of the National Academy of Sciences of the United States of America. 1974;**71**(4):1207-1210. PMID: 4208546

[89] Raines EW. PDGF and cardiovascular disease. Cytokine & Growth Factor Reviews. 2004;**15**(4):237-254. DOI: 10.1016/j.cytogfr.2004.03.004

[90] Osada-Oka M, Ikeda T, Imaoka S, Akiba S, Sato T. VEGF-enhanced proliferation under hypoxia by an autocrine mechanism in human vascular smooth muscle cells. Journal of Atherosclerosis and Thrombosis. 2008;**15**(1):26-33. DOI: 10.5551/jat.E533

[91] Rudijanto A. The role of vascular smooth muscle cells on the pathogenesis of atherosclerosis. Acta Medica Indonesiana. 2007;**39**(2):86-93. PMID: 17933075

[92] Kraemer R, Nguyen H, March KL, Hempstead B. NGF activates similar intracellular signaling pathways in vascular smooth muscle cells as PDGF-BB but elicits different biological responses. Arteriosclerosis, Thrombosis, and Vascular Biology. 1999;**19**(4):1041-1050. DOI: 10.1161/01.ATV.19.4.1041

[93] Ruiz-Torres A. The role of insulin-like growth factor 1 and insulin in ageing and atherosclerosis. Novartis Foundation Symposium. 2002;**242**:143-153; discussion 153-60. PMID: 11855685

[94] Jensen BA, Chemnitz J, Christensen BC, Junker P, Lorenzen I. D-penicillamine-induced angiopathy in rats. The effect of high dose D-penicillamine treatment on aortic permeability to albumin and on the ultrastructure of the vessel. Acta Pathologica, Microbiologica, et Immunologica Scandinavica. Section A. 1983;**91**(6):403-411. 6666578

[95] Smani Y, Faivre B, Fries I, Labrude P, Vigneron C. Potential mechanism of dextran-conjugated hemoglobin penetration inside arterial wall. Archives des Maladies du Coeur et des Vaisseaux. 2006;**99**(7-8):722-776. PMID: 17061452

[96] Sturge J, Carey N, Davies AH, Powell JT. Fibrin monomer and fibrinopeptide B act additively to increase DNA synthesis in smooth muscle cells cultured from human saphenous vein. Journal of Vascular Surgery. 2001;**33**(4):847-853. DOI: 10.1067/mva.2001.111984

[97] Isenberg JS, Calzada MJ, Zhou L, Guo N, Lawler J, Wang XQ, Frazier WA, Roberts DD. Endogenous thrombospondin-1 is not necessary for proliferation but is permissive for vascular smooth muscle cell responses to platelet-derived growth factor. Matrix Biology. 2005;**24**(2):110-123. DOI: 10.1016/j.matbio.2005.01.002

[98] Smiljanic K, Dobutovic B, Obradovic M, Nikolic D, Marche P, Isenovic ER. Involvement of the ADAM 12 in thrombin-induced rat's VSMCs proliferation. Current Medicinal Chemistry. 2011;**18**(22):3382-3386. DOI: 10.2174/092986711796504709

[99] Sprague AH, Khalil RA. Inflammatory cytokines in vascular dysfunction and vascular disease. Biochemical Pharmacology. 2009;**78**(6):539-552. DOI: 10.1016/j.bcp.2009.04.029

[100] Sai S, Iwata A, Thomas R, Allen MD. Vascular cell adhesion molecule-1 up-regulation and phenotypic modulation of vascular smooth muscle cells predate mononuclear infiltration in transplant arteriopathy. The Journal of Thoracic and Cardiovascular Surgery. 2001;**122**(3):508-517. DOI: 10.1067/mtc.2001.113601

[101] Hu Y, Xu Q. Adventitial biology: Differentiation and function. Arteriosclerosis, Thrombosis, and Vascular Biology. 2011;**31**(7):1523-1529. DOI: 10.1161/ATVBAHA.110.221176

[102] Schwartz CJ, Valente AJ, Sprague EA, Kelley JL, Suenram CA, Rozek MM. Atherosclerosis as an inflammatory process. The roles of the monocyte-macrophage. Annals of the New York Academy of Sciences. 1985;**454**:115-120. DOI: 10.1111/j.1749-6632.1985.tb11849.x

[103] Blum A, Miller HI. The role of inflammation in atherosclerosis. Israel Journal of Medical Sciences. 1996;**32**(11):1059-1065. 8960073

[104] Maxova H, Novotna J, Vajner L, Tomasova H, Vytasek R, Vizek M, Bacakova L, Valouskova V, Eliasova T, Herget J. In vitro hypoxia increases production of matrix metalloproteinases and tryptase in isolated rat lung mast cells. Physiological Research. 2008;**57**:903-910. PMID: 18052689

[105] Maxova H, Bacakova L, Lisa V, Novotna J, Tomasova H, Vizek M, Herget J. Production of proteolytic enzymes in mast cells, fibroblasts, vascular smooth muscle and endothelial cells cultivated under normoxic or hypoxic conditions. Physiological Research. 2010;**59**:711-719. PMID: 20406036

[106] Sung HJ, Eskin SG, Sakurai Y, Yee A, Kataoka N, McIntire LV. Oxidative stress produced with cell migration increases synthetic phenotype of vascular smooth muscle cells. Annals of Biomedical Engineering. 2005;**33**(11):1546-1554. DOI: 10.1007/s10439-005-7545-2

[107] Takahashi K, Takeya M, Sakashita N. Multifunctional roles of macrophages in the development and progression of atherosclerosis in humans and experimental animals. Medical Electron Microscopy. 2002;**35**(4):179-203. DOI: 10.1007/s007950200023

[108] Benditt EP, Benditt JM. Evidence for a monoclonal origin of human atherosclerotic plaques. Proceedings of the National Academy of Sciences of the United States of America. 1973;**70**(6):1753-1756. PMID: 4515934

[109] Pearson TA, Kramer EC, Solez K, Heptinstall RH. The human atherosclerotic plaque. The American Journal of Pathology. 1977;**86**(3):657-664. PMID: 842615

[110] Sjölund M, Madsen K, von der Mark K, Thyberg J. Phenotype modulation in primary cultures of smooth-muscle cells from rat aorta. Synthesis of collagen and elastin. Differentiation. 1986;**32**(2):173-180. DOI: 10.1111/j.1432-0436.1986.tb00570.x

[111] Steitz SA, Speer MY, Curinga G, Yang HY, Haynes P, Aebersold R, Schinke T, Karsenty G, Giachelli CM. Smooth muscle cell phenotypic transition associated with calcification: Upregulation of Cbfa1 and downregulation of smooth muscle lineage markers. Circulation Research. 2001;**89**(12):1147-1154. DOI: 10.1161/hh2401.101070

[112] Trion A, van der Laarse A. Vascular smooth muscle cells and calcification in atherosclerosis. American Heart Journal. 2004;**147**(5):808-814. DOI: 10.1016/j.ahj.2003.10.047

[113] Lin ME, Chen TM, Wallingford MC, Nguyen NB, Yamada S, Sawangmake C, Zhang J, Speer MY, Giachelli CM. Runx2 deletion in smooth muscle cells inhibits vascular osteochondrogenesis and calcification but not atherosclerotic lesion formation. Cardiovascular Research. 2016. pii: cvw205. DOI: 10.1093/cvr/cvw205

[114] Owens GK. Control of hypertrophic versus hyperplastic growth of vascular smooth muscle cells. The American Journal of Physiology. 1989;**257**(6 Pt 2):H1755-H1765. DOI: 10.1152/ajpheart.1989.257.6.H1755

[115] Hixon ML, Gualberto A. Vascular smooth muscle polyploidization--from mitotic checkpoints to hypertension. Cell Cycle. 2003;**2**(2):105-110. DOI: 10.4161/cc.2.2.341

[116] Nagata Y, Jones MR, Nguyen HG, McCrann DJ, St Hilaire C, Schreiber BM, Hashimoto A, Inagaki M, Earnshaw WC, Todokoro K, Ravid K. Vascular smooth muscle cell polyploidization involves changes in chromosome passenger proteins and an endomitotic cell cycle. Experimental Cell Research. 2005;**305**(2):277-291. DOI: 10.1016/j.yexcr.2004.12.028

[117] Bacakova L, Pellicciari C, Bottone MG, Lisa V, Mares V. A sex-related difference in the hypertrophic versus hyperplastic response of vascular smooth muscle cells to repeated passaging in culture. Histology and Histopathology. 2001;**16**(3):675-684. PMID: 11510955

[118] McCrann DJ, Yang D, Chen H, Carroll S, Ravid K. Upregulation of Nox4 in the aging vasculature and its association with smooth muscle cell polyploidy. Cell Cycle. 2009;**8**(6): 902-908. DOI: 10.4161/cc.8.6.7900

[119] Blaes N, Bourdillon MC, Daniel-Lamaziere JM, Michaille JJ, Andujar M, Covacho C. Isolation of two morphologically distinct cell lines from rat arterial smooth muscle expressing high tumorigenic potentials. In Vitro Cellular & Developmental Biology. 1991;**27A**(9):725-734. PMID: 1917792

[120] Papageorgiou P, Morgan KG. Intracellular free Ca2+ is elevated in hypertrophic aortic muscle from hypertensive rats. The American Journal of Physiology. 1991;**260**(2 Pt 2): H507-H515. DOI: 10.1152/ajpheart.1991.260.2.H507

[121] Jernigan NL, Resta TC, Gonzalez Bosc LV. Altered redox balance in the development of chronic hypoxia-induced pulmonary hypertension. Advances in Experimental Medicine and Biology. 2017;**967**:83-103. DOI: 10.1007/978-3-319-63245-2_7

[122] Orton EC, LaRue SM, Ensley B, Stenmark K. Bromodeoxyuridine labeling and DNA content of pulmonary arterial medial cells from hypoxia-exposed and nonexposed healthy calves. American Journal of Veterinary Research. 1992;**53**(10):1925-1930. PMID: 1456542

[123] Herget J, Novotna J, Bibova J, Povysilova V, Vankova M, Hampl V. Metalloproteinase inhibition by Batimastat attenuates pulmonary hypertension in chronically hypoxic rats. American Journal of Physiology. Lung Cellular and Molecular Physiology. 2003;**285**(1):L199-L208. DOI: 10.1152/ajplung.00167.2002

[124] Irwin DC, Baek JH, Hassell K, Nuss R, Eigenberger P, Lisk C, Loomis Z, Maltzahn J, Stenmark KR, Nozik-Grayck E, Buehler PW. Hemoglobin-induced lung vascular oxidation, inflammation, and remodeling contribute to the progression of hypoxic pulmonary hypertension and is attenuated in rats with repeated-dose haptoglobin administration. Free Radical Biology & Medicine. 2015;**82**:50-62. DOI: 10.1016/j.freeradbiomed.2015.01.012

[125] Morris CR. Asthma management: Reinventing the wheel in sickle cell disease. American Journal of Hematology. 2009;**84**(4):234-241. DOI: 10.1002/ajh.21359

[126] Racanelli AC, Kikkers SA, Choi AMK, Cloonan SM. Autophagy and inflammation in chronic respiratory disease. Autophagy. 2017:1-12. DOI: 10.1080/15548627.2017.1389823

[127] Dournes G, Laurent F, Coste F, Dromer C, Blanchard E, Picard F, Baldacci F, Montaudon M, Girodet PO, Marthan R, Berger P. Computed tomographic measurement of airway remodeling and emphysema in advanced chronic obstructive pulmonary disease. Correlation with pulmonary hypertension. American Journal of Respiratory and Critical Care Medicine. 2015;**191**(1):63-70. DOI: 10.1164/rccm.201408-1423OC

[128] Fein DG, Zaidi AN, Sulica R. Pulmonary hypertension due to common respiratory conditions: Classification, evaluation and management strategies. Journal of Clinical Medicine. 2016;**5**(9):E75. DOI: 10.3390/jcm5090075

[129] Quarck R, Wynants M, Ronisz A, Sepulveda MR, Wuytack F, Van Raemdonck D, Meyns B, Delcroix M. Characterization of proximal pulmonary arterial cells from chronic thromboembolic pulmonary hypertension patients. Respiratory Research. 2012;**13**:27. DOI: 10.1186/1465-9921-13-27

[130] Chen L, Li YS, Cui J, Ning JN, Wang GS, Qian GS, Lu KZ, Yi B. MiR-206 controls the phenotypic modulation of pulmonary arterial smooth muscle cells induced by serum from rats with hepatopulmonary syndrome by regulating the target gene, annexin A2. Cellular Physiology and Biochemistry. 2014;**34**(5):1768-1779. DOI: 10.1159/000366377

[131] Hampl V, Herget J. Role of nitric oxide in the pathogenesis of chronic pulmonary hypertension. Physiological Reviews. 2000;**80**(4):1337-1372. DOI: 10.1152/physrev.2000.80.4.1337

[132] Stenmark KR, Fagan KA, Frid MG. Hypoxia-induced pulmonary vascular remodeling: Cellular and molecular mechanisms. Circulation Research. 2006;**99**(7):675-691. DOI: 10.1161/01.RES.0000243584.45145.3f

[133] Stenmark KR, Frid MG, Graham BB, Tuder RM. Dynamic and diverse changes in the functional properties of vascular smooth muscle cells in pulmonary hypertension. Cardiovascular Research. 2018;**114**(4):551-564. DOI: 10.1093/cvr/cvy004

[134] Gui Y, He GH, Walsh MP, Zheng XL. Predisposition to tetraploidy in pulmonary vascular smooth muscle cells derived from the Eker rats. American Journal of Physiology. Lung Cellular and Molecular Physiology. 2007;**293**(3):L702-L711. DOI: 10.1152/ajplung.00016.2007

[135] Stenmark KR, Tuder RM, El Kasmi KC. Metabolic reprogramming and inflammation act in concert to control vascular remodeling in hypoxic pulmonary hypertension. Journal of Applied Physiology (1985). 2015;**119**(10):1164-1172. DOI: 10.1152/japplphysiol.00283.2015

[136] Stenmark KR, Bouchey D, Nemenoff R, Dempsey EC, Das M. Hypoxia-induced pulmonary vascular remodeling: Contribution of the adventitial fibroblasts. Physiological Research. 2000;**49**(5):503-517. PMID: 11191356

[137] Leroux-Berger M, Queguiner I, Maciel TT, Ho A, Relaix F, Kempf H. Pathologic calcification of adult vascular smooth muscle cells differs on their crest or mesodermal embryonic origin. Journal of Bone and Mineral Research. 2011;**26**(7):1543-1553. DOI: 10.1002/jbmr.382

[138] Hsu S, Chu JS, Chen FF, Wang A, Li S. Effects of fluid shear stress on a distinct population of vascular smooth muscle cells. Cellular and Molecular Bioengineering. 2011;**4**(4):627-636. DOI: 10.1007/s12195-011-0205-8

[139] Gadson PF Jr, Rossignol C, McCoy J, Rosenquist TH. Expression of elastin, smooth muscle alpha-actin, and c-Jun as a function of the embryonic lineage of vascular smooth muscle cells. In Vitro Cellular & Developmental Biology. Animal. 1993;**29A**(10):773-781. PMID: 8118612

[140] Yamamoto M, Yamamoto K, Noumura T. Type I collagen promotes modulation of cultured rabbit arterial smooth muscle cells from a contractile to a synthetic phenotype. Experimental Cell Research. 1993;**204**(1):121-129. DOI: 10.1006/excr.1993.1016

[141] Karnik SK, Brooke BS, Bayes-Genis A, Sorensen L, Wythe JD, Schwartz RS, Keating MT, Li DY. A critical role for elastin signaling in vascular morphogenesis and disease. Development. 2003;**130**(2):411-423. DOI: 10.1242/dev.00223

[142] Lange M, Fujikawa T, Koulova A, Kang S, Griffin MJ, Lassaletta AD, Erat A, Tobiasch E, Bianchi C, Elmadhun N, Sellke FW, Usheva A. Arterial territory-specific phosphorylated retinoblastoma protein species and CDK2 promote differences in the vascular smooth muscle cell response to mitogens. Cell Cycle. 2014;**13**(2):315-323. DOI: 10.4161/cc.27056

[143] Faries PL, Rohan DI, Wyers MC, Marin ML, Hollier LH, Quist WC, LoGerfo FW. Vascular smooth muscle cells derived from atherosclerotic human arteries exhibit greater adhesion, migration, and proliferation than venous cells. The Journal of Surgical Research. 2002;**104**(1):22-28. DOI: 10.1006/jsre.2002.6399

[144] Xiao Y, Huang Z, Yin H, Zhang H, Wang S. Desmuslin gene knockdown causes altered expression of phenotype markers and differentiation of saphenous vein smooth muscle cells. Journal of Vascular Surgery. 2010;**52**(3):684-690. DOI: 10.1016/j.jvs.2010.03.069

[145] Filova E, Parizek M, Olsovska J, Kamenik Z, Brynda E, Riedel T, Vandrovcova M, Lisa V, Machova L, Skalsky I, Szarszoi O, Suchy T, Bacakova L. Perivascular sirolimus-delivery system. International Journal of Pharmaceutics. 2011;**404**(1-2):94-101. DOI: 10.1016/j.ijpharm.2010.11.005

[146] Skalský I, Szárszoi O, Filová E, Pařízek M, Lytvynets A, Malušková J, Lodererová A, Brynda E, Lisá V, Burdíková Z, Capek M, Pirk J, Bačáková L. A perivascular system releasing sirolimus prevented intimal hyperplasia in a rabbit model in a medium-term study. International Journal of Pharmaceutics. 2012;**427**(2):311-319. DOI: 10.1016/j.ijpharm.2012.02.023

[147] Durante W. Role of arginase in vessel wall remodeling. Frontiers in Immunology. 2013;**4**:111. DOI: 10.3389/fimmu.2013.00111

[148] Imegwu OJ, Entersz I, Graham AM, Nackman GB. Heterotypic smooth muscle cell/endothelial cell interactions differ between species. The Journal of Surgical Research. 2001;**98**(2):85-88. DOI: 10.1006/jsre.2001.6165

[149] Rosen EM, Goldberg ID, Shapiro HM, Levenson SE, Halpin PA, Faraggi D. Strain and site dependence of polyploidization of cultured rat smooth muscle. Journal of Cellular Physiology. 1986;**128**(2):337-344. DOI: 10.1002/jcp.1041280228

[150] Bacakova L, Kunes J. Gender differences in growth of vascular smooth muscle cells isolated from hypertensive and normotensive rats. Clinical and Experimental Hypertension. 2000;**22**(1):33-44. PMID: 10685723

[151] Bacakova L, Mares V. Cell kinetics of aortic smooth muscle cells in long-term cultures prepared from rats raised under conventional and SPF conditions. Physiological Research. 1995;**44**(6):389-398. PMID: 8798274

[152] Thyberg J. Differentiated properties and proliferation of arterial smooth muscle cells in culture. International Review of Cytology. 1996;**169**:183-265. PMID: 8843655

[153] van Neck JW, van Berkel PH, Telleman P, Steijns LS, Onnekink C, Bloemers HP. Effect of ploidy on transcription levels in cultured rat aortic smooth muscle cells. FEBS Letters. 1992;**297**(1-2):189-195. DOI: 10.1016/0014-5793(92)80358-N

[154] Werstiuk ES, Lee RM. Vascular beta-adrenoceptor function in hypertension and in ageing. Canadian Journal of Physiology and Pharmacology. 2000;**78**(6):433-452. PMID: 10914632

[155] Li M, Fukagawa NK. Age-related changes in redox signaling and VSMC function. Antioxidants & Redox Signaling. 2010;**12**(5):641-655. DOI: 10.1089/ars.2009.2854

[156] Martín-Pardillos A, Sorribas V. Effects of donor age and proliferative aging on the phenotype stability of rat aortic smooth muscle cells. Physiological Reports. 2015;**3**(11). DOI: 10.14814/phy2.12626

[157] Uryga AK, Bennett MR. Ageing induced vascular smooth muscle cell senescence in atherosclerosis. The Journal of Physiology. 2016;**594**(8):2115-2124. DOI: 10.1113/JP270923

[158] Travo P, Barrett G, Burnstock G. Differences in proliferation of primary cultures of vascular smooth muscle cells taken from male and female rats. Blood Vessels. 1980;**17**(2): 110-116. PMID: 7362876

[159] Bacakova L, Lisa V, Pellicciari C, Mares V, Bottone MG, Kocourek F. Sex related differences in the adhesion, migration, and growth of rat aortic smooth muscle cells in culture. In Vitro Cellular & Developmental Biology. Animal. 1997a;**33**(6):410-413. DOI: 10.1007/s11626-997-0055-9

[160] Bacakova L, Mares V, Lisa V. Gender-related differences in adhesion, growth and differentiation of vascular smooth muscle cells are enhanced in serum-deprived cultures. Cell Biology International. 1999;**23**(9):643-648. DOI: 10.1006/cbir.1999.0417

[161] Bacakova L, Pellicciari C, Bottone MG, Lisa V, Mares V. A sex-related difference in the hypertrophic versus hyperplastic response of vascular smooth muscle cells to repeated passaging in culture. Histology and Histopathology. 2001;**16**(3):675-684. PMID: 11510955

[162] Bacakova L, Mares V, Lisa V, Kocourek F. Sex-dependent differences in growth and morphology of cultured vascular smooth muscle cells from newborn rats. Physiological Research. 1997b;**46**(5):403-406. PMID: 9728488

[163] Ely DL, Falvo J, Dunphy G, Caplea A, Salisbury R, Turner M. The spontaneously hypertensive rat Y chromosome produces an early testosterone rise in normotensive rats. Journal of Hypertension. 1994;**12**(7):769-774. PMID: 7963505

[164] Ely D, Caplea A, Dunphy G, Daneshvar H, Turner M, Milsted A, Takiyyudin M. Spontaneously hypertensive rat Y chromosome increases indexes of sympathetic nervous system activity. Hypertension. 1997;**29**(2):613-618. DOI: 10.1161/01.HYP.29.2.613

IntechOpen