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Chapter 2

Vascular Wall-Resident Multipotent Stem Cells within the Process of Vascular Remodelling

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Additional information is available at the end of the chapter

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Abstract

Processes of new vessel formation are central events in tissue development and repair. Therein, sprouting endothelial cells and/or endothelial progenitor cells form immature blood vessels that lack coverage by pericytes and other mural cells. Subsequently, vascular remodelling takes place, in which association with mural cells (pericytes and smooth muscle cells, SMC) stabilizes these immature vessels resulting in normalization of the vascular structures. Vascular remodelling is a dynamic and strictly regulated process; an ordered remodelling seems to be critical for proper vascular development, maintenance and stability of the vessel wall. The molecular and cellular changes associated with this process and its importance for tumour growth remain elusive. Up to now, the origin of vascular wall cells in tumours and the molecular mechanisms that govern their recruitment and association with angiogenic endothelial cells (vascular stabilization) are not well understood. There is some evidence that pericytes and SMC might originate from multipotent mesenchymal stem cells. This chapter aims to explore the role of tissue-resident multipotent stem cells of mesenchymal nature (VW-MPSCs) which putatively reside in the adventitia of adult blood vessels within the process of vascular remodelling of tumour blood vessels as well as of molecular factors that regulate VW-MPSC differentiation into pericytes and SMC.

Keywords: Vascular wall-resident multipotent stem cell, vascular remodelling, vascular stabilization, tumour vascularization, postnatal vasculogenesis

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1. Introduction

This chapter provides a summary of the current literature addressing the importance of vascular wall-resident multipotent stem cells within the process of vascular remodelling. First, the role of pericytes and smooth muscle cells (SMC) causing stabilization of angiogenic tumour vessels will be discussed at the molecular and cellular level. This stabilization phase is crucial for the survival of newly formed vessels, as immature vessels may rapidly become subject to regression and cell death when the angiogenic stimulus is removed. The second part of the chapter will focus on vascular wall-resident multipotent stem cells and evaluate the contribution of circulating progenitor cells versus vessel-resident stem cells in the generation of pericytes and SMC within the neovascularization process. Here, the hypothesis will be proved that tissue-resident multipotent stem cells which putatively reside within the vascular adventitia, rather than circulating multipotent stem cells, are the major source for pericytes and SMC in the vascular stabilization processes. Finally, the regulation of differentiation of vascular wall-resident multipotent stem cells into SMC will be discussed.

Aspects of vascular stabilization, e.g., some decisive factors for the mobilization of vessel-resident stem cells and differentiation into pericytes and SMC, may have the potential for clinically relevant applications in themselves. A better understanding of the molecular processes in these cells could lead to the identification of new therapeutic targets.

2. Pericytes and smooth muscle cells cause a stabilization of newly formed tumour vessels

Endothelia cover the innermost cell layer of the blood vessels. This continuous endothelium is made impermeable for substances dissolved in the blood by the formation of tight junctions in a first approximation. The necessary exchange of substances between blood and tissues is tightly controlled by a highly selective transport mechanism [1]. The uncontrolled cell growth which prevails in tumours results in a relative disparity between the tumour tissue and the sufficient formation of vascular structures. The initiation of tumour angiogenesis is associated with a structural destabilization of existing blood vessels. This causes an abnormally increased vascular permeability, i.e., the existing endothelium is fenestrated, and endothelial cells lose contact with one another and the underlying basal lamina. Finally, contact with the surrounding mural, peri-endothelial cells (pericytes for capillaries and SMC of larger blood vessels) is lost. This leads to the fact that the now mature and quiescent endothelial cells start to migrate and proliferate [2-4]. During angiogenesis, continuous endothelial cells (the particularly impermeable form of the endothelial cells) undergo phases where they are not continuous, so are discontinuous (angiogenic endothelial cells). Chemotactic stimuli and vascular active growth factors such as VEGF (vascular endothelial growth factor) and bFGF (basic fibroblast growth factor) secreted by tumour cells induce mobilization and migration of angiogenic endothelial cells towards the tumour cells, which then build up new small blood vessels [5, 6].

During tumour angiogenesis, the hierarchical order of the blood vessels in large, medium and small blood vessels that is found in normal vasculature is lost. New vessel formation in the

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tumour is disordered in structure; chaotic vascular structures are formed with areas of apparent excess supply, in addition to areas with an undersupply of blood and in particular oxygen and nutrition. In addition, tumour vessels have peculiarities in their structure: tumour vessels run tortuously in the tissue, may end blindly (increased permeability of blood vessels), have arteriovenous shunts (shorting connections causing liquid transfer between normally separate vessels), or be directed opposite to the blood flow (heterogeneous perfusion of the tumour tissue). The endothelial lining is incomplete [4, 7, 8]. The newly formed vessel walls lack the smooth muscle elements in their walls, so that they cannot actively respond to physiological stimuli. For this reason, the newly formed angiogenic capillaries bear an increased risk of rupture. Because of these features, tumour vessels prove to be functionally inferior. This complicates the efficient administration of intravenous drugs in cancer therapy [9-12].

While angiogenesis describes new vessel formation by endothelial cells derived from pre-existing vessels, postnatal vasculogenesis denotes vessel formation by assembly of endothelial and/or vascular progenitor cells [13, 14]. Thus, the active cellular component in these processes is granted by endothelial lineage cells, but neovascularization does not only depend on endothelial cell migration and proliferation with subsequent formation of endothelial tubes; it also requires pericyte coverage of vascular sprouts for vessel stabilization [15-18]. Thereby, the vascular network can mature by recruitment of pericytes as well as SMC to stabilize the immature tumour vessels (Figure 1).

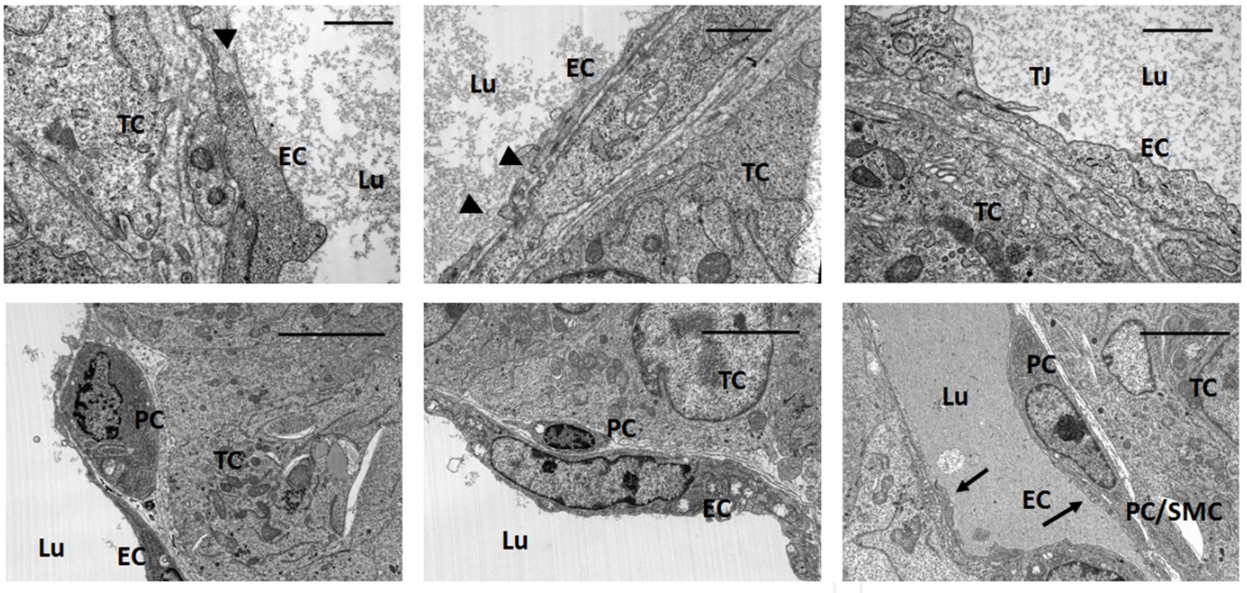


Figure 1. Ultrastructural analysis of angiogenic tumour vessels. Subcutaneously grown B16F10 melanoma tumours were removed 28 days after tumour induction and subjected to electron microscopic analysis. The presence of fenestrae (emphasized by arrowheads) in angiogenic endothelial cells (EC) corroborate the less mature and functional inferior phenotype of these tumour vessels. Upon vessel maturation, these fenestrae disappear (upper panel). Vascular remodelling can be further observed by association and integration of pericytes to the newly formed blood vessels, resulting in vascular stabilization and thus maturation of angiogenic endothelial cells (lower panel). At the structural level, the recruited pericytes are assembled into new capillaries and change cell morphology into a more flattened, smooth muscle cell-like phenotype. In some tumour vessels, vascular mural cells seem to be more regularly integrated into the wall of the new capillaries because of their tight contact with the endothelial cells, shown, for example, in their sharing the same basement membrane, thereby indicating vessel stabilization and maturation (arrows). On PC pericyte: SMC smooth muscle cell, TC tumour cell, Lu lumen. Scale bar 1μm upper panel, 5μm lower panel.

At the molecular level, for the expression of important signalling molecules or receptors, or cell adhesion molecules, there is a locally pronounced heterogeneity in the tumour vascular bed [19, 20]. For a long time, these findings were interpreted as if there were no restructuring processes (vascular remodelling) of newly formed blood vessels in terms of a re-stabilization in the tumour vascular bed. Recent findings, however, show that even tumour vessels undergo a reorganization in terms of vascular stabilization to a certain degree [21, 22]. Electron microscopy analyses indicate that partially stabilized blood vessels exist that differ in their architecture from the usual blood vessels next to structurally stabilized and mature blood vessels, so that angiogenic and less stabilized vessels are disordered and regarded as immature. In combination with the fact that tumours require blood vessels for progressive tumour growth, many new cancer therapies directed against the tumour vasculature (anti-vascular agents, anti-angiogenic agents) have been investigated. It was thought that these anti-angiogenic therapies could destroy the tumour vasculature to deprive the tumour of oxygen and nutrients. By contrast, it was shown that the process of vascular remodelling in tumours was affected during treatment with angiogenesis inhibitors [18, 23-26]. Besides a dramatic tumour regression observed some angiogenesis inhibitors, the tumours also became resistant to prolonged anti-angiogenic therapy. The tumour regression was histological, revealing a reduction in tumour vascularity observed during treatment predominantly as a result of the loss of less mature and highly proliferative small-calibre vessels. The remaining vessels were characterized by an increase in vessel diameter, and by the association and integration of pericytes and SMC leading to vascular stabilization in terms of vessel maturation, and thus a normalization of the vascular. This finally leads to an alternative hypothesis, that certain anti-angiogenic agents can also transiently normalize the abnormal structure and function of tumour vasculature to make it more efficient for oxygen and drug delivery [26]. Meanwhile, an extensive arsenal of anti-angiogenic compounds is available, and their effectiveness is currently being tested in numerous clinical studies. Bevacizumab is a humanized monoclonal anti-VEGF antibody which neutralizes any VEGF isoforms and prevents the interaction of VEGF with the corresponding receptors [27-29]. Clinical trials with bevacizumab show synergistic anti-tumour and chemotherapeutic effects. The results of histological examination of tumour tissue in clinical trials with anti-angiogenic substances showed a stabilization of tumour vessels, which was associated with a reduction in vascular density in the tumour tissue [11, 30-32].

In general, bevacizumab is used as first-line drug in combination with conventional chemotherapeutics in patients with metastatic colorectal cancer, unless contra-indicated. The continuation of bevacizumab beyond first-line progression is still controversial, due to a lack of prospective randomized evidence in this setting [33]. The clinical efficacy of angiogenesis inhibitors targeting vascular endothelial cells has not been as successful as initially hoped, and improved clinical outcomes have been observed in combination with chemotherapy or additional drugs for many types of human cancer. This may be at least partially due to the fact that anti-angiogenic therapy triggers vascular stabilization including pericyte coverage, and that pericyte coverage further impairs tumour vessel regression in response to anti-angiogenic treatment [34]. Furthermore, tumour vessels which are resistant to anti-angiogenic therapy are characterized by an increase in vessel diameter and a normalization of vascular structures.

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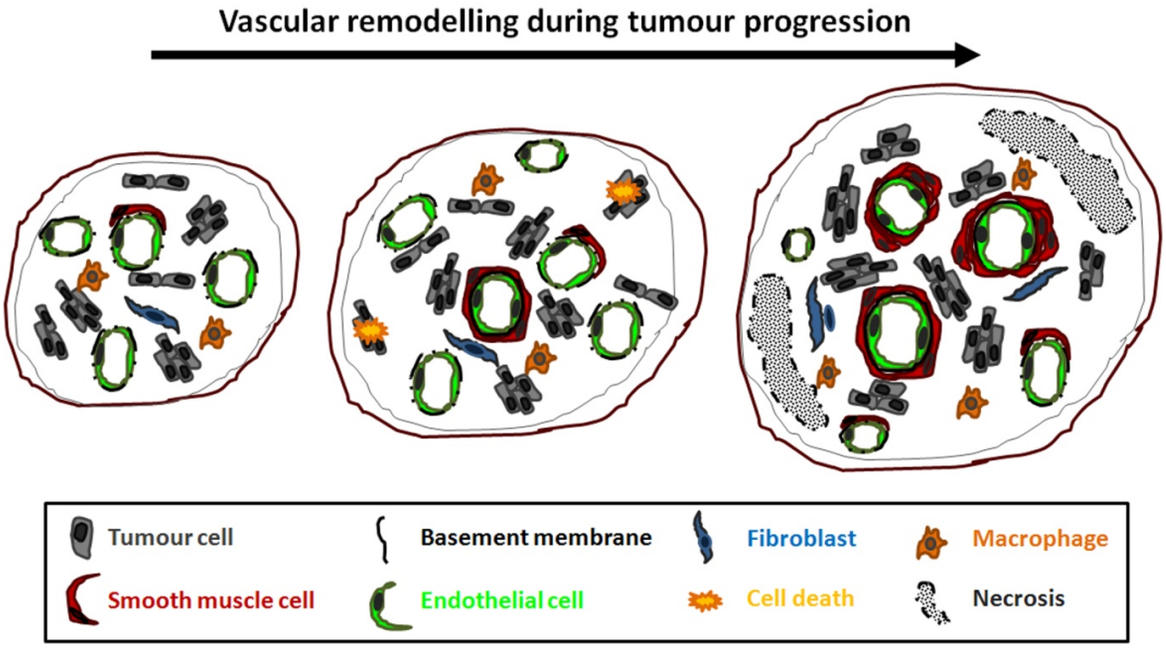


Figure 2. Scheme of the extensive remodelling of the tumour vascular bed by a partial structural stabilization of blood vessels upon tumour progression. Newly formed tumour vessels supply a highly dense network of immature and unstable vessels. Upon tumour progression, angiogenic vessels can mature by the association and integration of pericytes and smooth muscle cells preferentially in the tumour centre zone. This process is accelerated in tumour therapy when anti-angiogenic agents are applied. Further vessel maturation leads to vessels characterized by an increased diameter, a reduction of vascular density and mural cell integration, resulting in normalization of the vascular bed. This process is accompanied by an extensive necrosis of the neighbouring tumour areas while viable tumour cell rows are circularly arranged around stabilized large arteries.

This normalization is achieved by the recruitment and integration of mature pericytes in the vessel wall for capillaries as well as SMC for larger vessels (Figure 2). This process is accelerated in tumour therapy when agents that affect the formation of new vessels (anti-angiogenic agents) were applied [11]. In contrast, the presence of VEGF led to ablation of pericyte coverage on nascent vascular sprouts and vessel destabilization [35]. Thus, targeting pericyte recruitment, coverage and function in addition to endothelial cells may be suitable for promoting progress in anti-angiogenic tumour therapy [36, 37]. In addition, the use of angiogenesis inhibitors which lead to a normalization of tumour vessels in combination with conventional therapies such as radiation or chemotherapy should lead to enhanced efficacy of drug delivery and diminished toxicity [38-40].

3. Vascular wall-resident multipotent stem cells stabilize angiogenic tumour blood vessels by differentiation into pericytes and smooth muscle cells

Until some years ago, the bone marrow and endothelial cell compartment lining the vessel lumen (sub-endothelial space) were thought to be the only sources providing vascular

progenitor cells. Results published recently have identified the human vessel wall as a niche for stem cells [41-44]. Herein, the blood vessels themselves harbour progenitors and multipotent stem cells (vascular wall-resident EPCs, VW-EPCs and haematopoietic stem cells, HPCs), clearly indicating the presence of stem cell niches outside the bone marrow and the peripheral blood [45-48]. Arterial vessels have what is termed an adventitial regeneration zone, in which those various stem and progenitor cells reside (Figure 3). These cells are able to form vascular networks and are capable of differentiating into endothelial cells and CD68+ macrophages [43, 46, 49]. However, the blood vessel wall is made not only of endothelial but also peri-endothelial cells (pericytes/SMC) and adventitial cells. Thus, the adequate formation of new blood vessels under hypoxia, during ischaemia or in tumour neovascularization, depends on the presence and recruitment of these peri-endothelial in addition to endothelial cells. Accordingly, the stem cell niche "vasculogenic zone" also harbours mesenchymal stem cells (MSCs) [43, 46].

Pericytes play a central role in tumour angiogenesis and these cells significantly affect the success of anti-angiogenic therapies. Thus it is important to identify pericytes in different tumour entities [50, 51]. In capillaries, pericytes are in close contact with endothelial cells and share the same capillary basement membrane. Pericytes express alpha-smooth muscle actin (ACTA2) and thus they might have contractile properties. However, the origins of pericytes and of SMC in tumours, and the molecular mechanisms that govern their recruitment and association with tumour vessels, are not clear. Endothelial expression of the platelet-derived growth factor B (PDGF-B) was shown to trigger the recruitment of pericytes necessary for the remodelling of newly formed vessels in terms of vascular stabilization, so that immature vessels with or without pericytes are formed [52]. Using an 'in vitro angiogenesis' system, Nicosia and co-workers suggested that pericytes are formed by migration and de-differentiation of arterial SMC [53]. Interestingly, pericytes have been assumed to differentiate in situ from mesenchymal cells [54]. In line with the idea that pericytes might have their origin in MSCs, it has been shown that Sca-1-positive bone marrow (BM)-derived cells are recruited to the site of tumour progression using the RIP-Tag2 model of pancreatic cancer [55].

In line with these findings, several studies identified human vascular wall-resident CD44+ multipotent stem cells (VW-MPSCs) within the adult human vascular adventitia which were capable of differentiation into pericytes and SMC [45, 46, 56-59]. VW-MPSCs were shown to contribute to in vivo vessel morphogenesis by co-implantation of isolated VW-MPSCs and human umbilical cord vein endothelial cell (HUVEC) in a matrigel plug assay [46]. Within the plugs, implanted HUVEC formed blood perfused vessels. Co-implanted VW-MPSCs assembled at the new vessels and were differentiated into transgelin-positive/ACTA2-positive SMC/pericytes, undoubtedly confirming that VW-MPSCs have the capability to differentiate into pericyte/SMC and thus contribute to morphogenesis of new vessels under in vivo conditions. Electron microscopic analysis further demonstrated at the ultrastructural level that VW-MPSCs were not only aligned to new capillaries but were also regularly integrated into the wall of new capillaries; for example, EC and pericytes are enclosed by the same basal lamina. Thus, a crucial hypothesis concerning the vessel-resident stem cells is that these cells are the "first-line" cells, which are available on the basis of their anatomic location as the first point of contact for tumour cells and for tumour cell-secreted factors [43, 46, 60, 61]. Moreover, it is hypothesized that MPSCs or smooth muscle progenitors, resident in the vessel wall, would serve as a source for local recruitment of cells to stabilize new immature vessels constructed

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only by endothelial cells. Under vascular restructuring processes (remodelling) these VW-MPSCs associate with the newly formed blood vessels of the tumour and differentiate into pericytes and SMC, which results in a stabilization of the newly formed vessels (Figure 3).

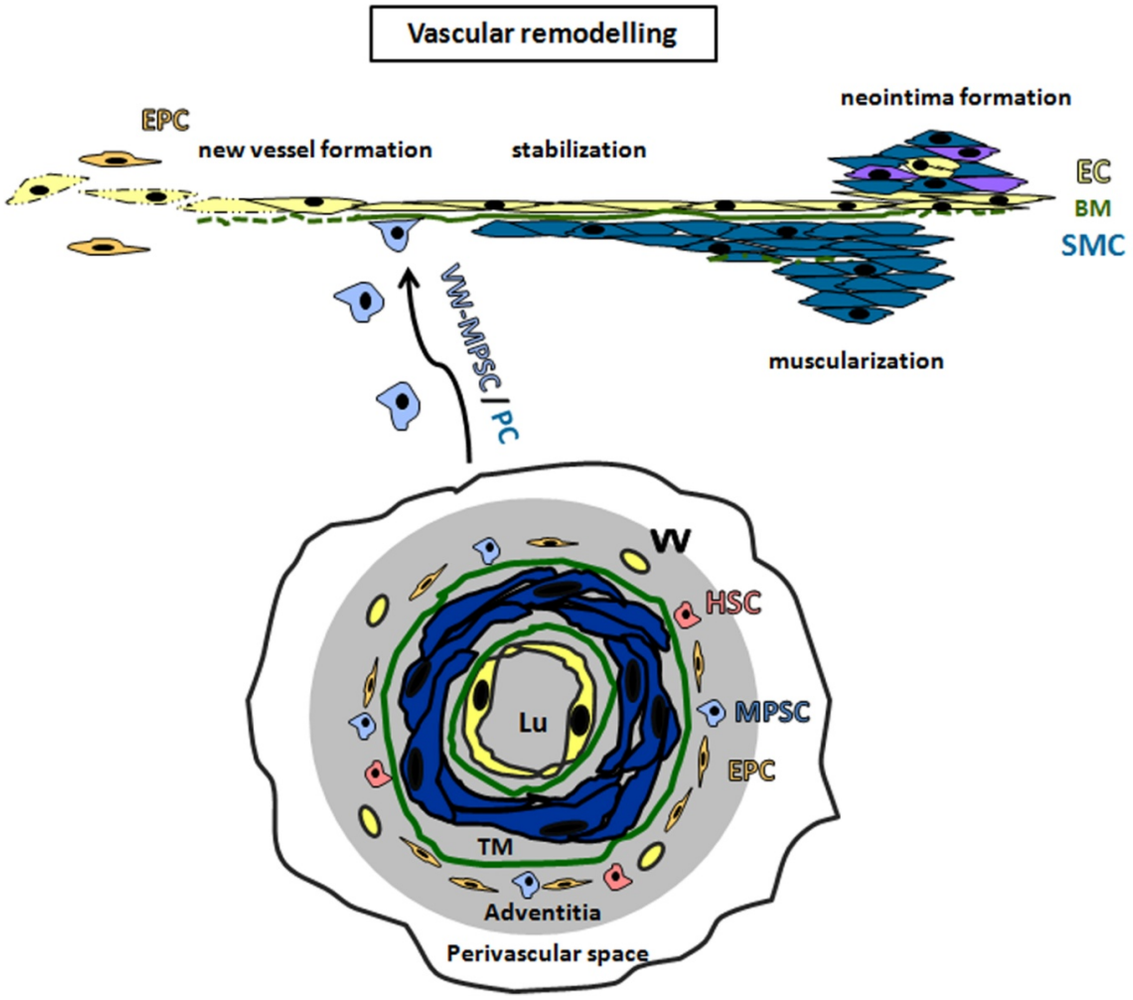


Figure 3. Vascular remodelling. New vessel formation by both angiogenesis and postnatal vasculogenesis is a prerequisite for tissue regeneration and several diseases including tumour progression and atherosclerosis. Vascular stabilization is achieved by the recruitment and integration of mature pericytes in the vessel wall for capillaries, as well as smooth muscle cells (SMC) for larger vessels. Intima, media (TM) and adventitia with vasa vasorum (VV) are fixed layers of the wall of large arteries and veins. The border between media and adventitia is marked by outer elastic membrane (green). The vasculogenic zone is a vascular mural zone located within the adventitia and close to the tunica media which harbours different subsets of vascular wall stem cells. The central hypothesis concerning vascular wall-resident multipotent MSCs (VW-MPSC) is that these cells serve as major source for pericytes and SMC for stabilization of new vessels, or repair of pre-existing vessels under physiological conditions. Localized within the vascular adventitia, which serves as an interface between the inner parts of the vessel wall, including blood flow and the surrounding tissue, the VW-MPSCs might serve as an important therapeutic target. Under vascular restructuring processes (remodelling), these MSCs associate with the newly formed blood vessels of the tumour and differentiate into pericytes and SMC, which results in a stabilization and thus normalization of angiogenic tumour blood vessels. VW-MPSCs' differentiation into SMC may be induced by tumours, inflammation and hypoxia in tissue areas around blood vessels, contributing to morphogenesis of new vessel walls (e.g., tumour vascularization, intimal lesions or neointima formation). In contrast to the direct action of MPSCs during tumour progression through becoming mobilized from their niche and subsequently differentiated at the site of injury, the protective effect of endogenous or exogenous applied MSCs could also be related to the modulation of paracrine characteristics of these cells. HSP, haematopoietic stem cell; EPC, endothelial progenitor cell; PC, pericyte; yellow, endothelial cells; green basement membrane and elastic membrane; blue, SMC.

Multipotent MSCs were intensively analysed using in vitro studies: optimized conditions were identified for their expansion and potential for differentiation along mesodermal lineages, e.g., into bone, fat, muscle and cartilage [62-65]. A frequently used source of MSCs is the bone marrow [66]. Here, only 0.01 to 0.001 % of the mononuclear cells in the BM are MSCs. Furthermore, human MSCs (hMSCs) can be obtained from umbilical cord blood, placental blood, foetal liver and adipose tissue [67-71]. It is further hypothesized that so-called permanent tissue stem cells exist in virtually every tissue type [72, 73]. In view of the fact that the blood vessels' area is a common structure of all tissues and organs, it is obvious that vessel-resident stem and progenitor cells may have great potential in biomedicine [46, 59, 74-76]. Together with the fact that tissue-specific stem cells differentiate predominantly into the tissue type from which they derive, vessel-resident (multipotent) MSCs may be particularly well suited to contribute to the formation of new vessels.

In general, abnormal vasculature is a hallmark of solid tumours. The exact quantification of tumour vessels is useful to evaluate prognosis, because the degree of angiogenesis is associated with tumour aggressiveness and clinical outcome [77]. Together with the fact that pericytes and SMC play a central role in vascular remodelling of tumour vessels, their recruitment and stable integration into stabilized tumour vessels may determine the success of anti-angiogenic therapies [78, 79]. Accordingly, future therapies targeting both endothelium and pericytes may favour progress in anti-angiogenic treatment for malignant tumours [80]. Thus, it is important to identify the origin and localization of pericytes and SMC in tumour tissues from cancer patients to gain a better understanding of their role in tumour growth and metastasis as well as to improve the outcome of anticancer therapies. Concerning the hypothesis that multipotent stem cells of mesenchymal nature (MPSCs) which express the (neural) stem cell marker nestin are the major source for pericytes and SMC in vascular stabilization processes, nestin-GFP transgenic mice were used in order to track MPSCs' contribution to the vascular remodelling processes. Nestin-GFP transgenic mice express GFP under the regulatory elements of the nestin promoter [81]. For transgene construction, the second intron of nestin gene was utilized, which was known to drive the expression in neural stem and progenitor cells. Furthermore, the 5′ upstream region (promoter region) in the transgene construct was included, the regulatory function of which is still unclear [82, 83]. Thus, these mice were ideally suited for the evaluation of the role of nestin-positive cells during the vascular remodelling of tumour blood vessels (Figure 4). Besides this, the BM tissue-resident nestin-GFP-positive cells are localized in the wall of mouse aortas and express nestin while lacking CD34 expression. Using arterial slice cultures of ex vivo isolates, these cells can be mobilized from their niche by factors secreted from cultured tumour cell lines, and are capable of differentiating into pericytes and SMC. In line with these results, Lin et al. have shown that tissue-resident MPSCs isolated from different anatomic locations gain the capacity to modulate the formation of vasculature by tightly surrounding newly formed microvessels as perivascular cells using a matrigel plug assay [84]. Furthermore, it has been demonstrated that human MPSCs derived either from the vascular adventitia or the bone marrow efficiently stabilized nascent blood vessels in vitro by functioning as perivascular precursor cells [46, 85]. Furthermore, vascular wall-resident nestin-GFP-positive cells can be isolated and cultivated. Primary cell cultures exhibited typical MSC characteristics. According to the guidelines, clonally expanded cells adhered on plastic,

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differentiated into adipocytes, chondrocytes and osteocytes under certain cell culture conditions [86]. These findings are in line with previous reports. Recently it has been elegantly demonstrated that nestin-GFP-positive cells in the BM are enriched in mesenchymal stem cell activities and are pericyte-like [87].

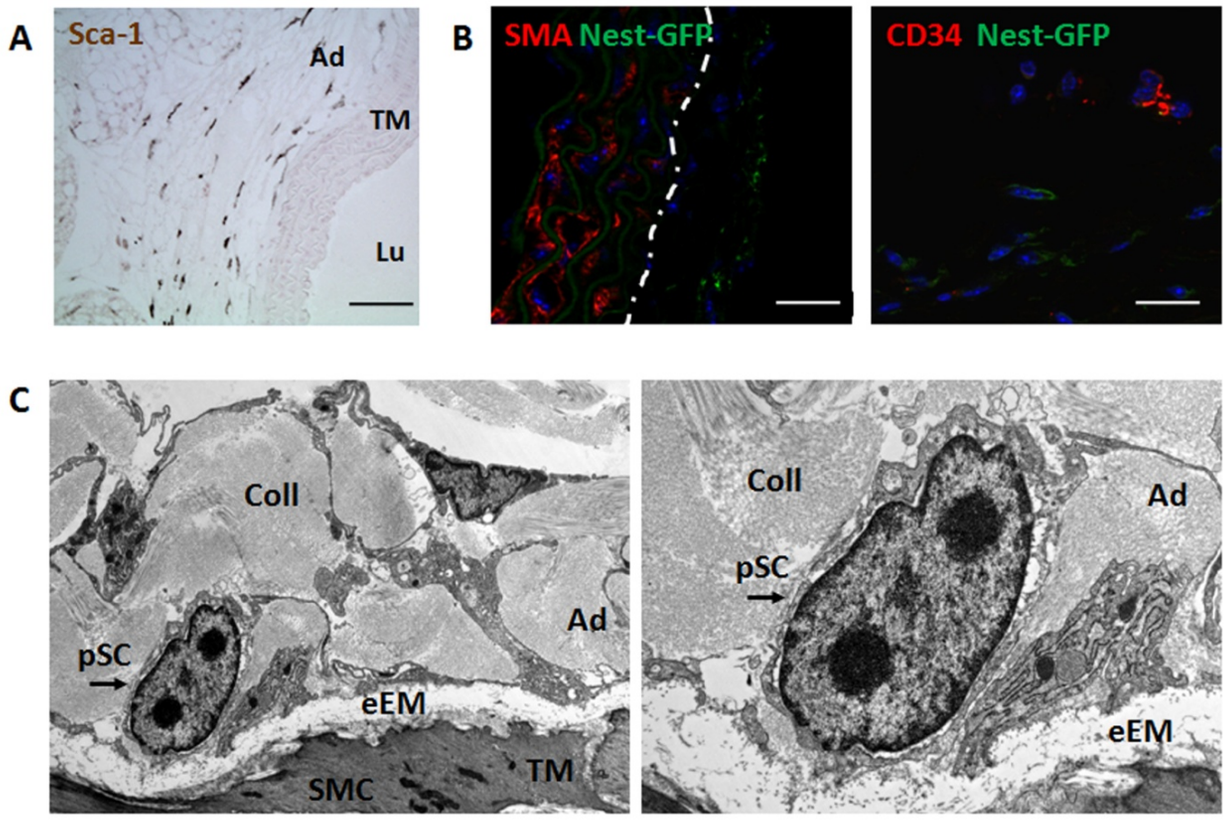


Figure 4. Nestin-GFP(+) multipotent cells are localized in the vasculogenic zone of murine aorta. (A) Immunohistological analysis of stem cell antigen-1 (Sca-1) expression in mouse aorta sections. Scale bar 100μm. (B) Immunofluorescence analysis of nestin-GFP-positive MSCs in their native niche was performed using double immunostainings on mouse aorta sections combining antibodies against GFP (green) and SMA or CD34 (red). Dotted line marks the border between media and adventitia of the aortic wall. Scale bar 20μm. (C) Electron microscopic analysis indicates the presence of undifferentiated cells (putative stem cells (pSC) in the vasculogenic zone (Ad) of the adventitia. eEM external elastic membrane, SMC smooth muscle cell, TM, tunica media, Coll collagen.

In order to determine the contribution of the tissue-resident MPSCs to the formation of tumour neovasculature, BM transplantation experiments were performed. Tissue-derived cells were tracked when wild-type BM cells were isolated from C57BL/6 mice and transplanted into lethally irradiated, age-matched, syngeneic, nestin-GFP transgenic recipients [81]. Tumours grown in reconstituted nestin-GFP transgenic mice which received wild-type BM showed that ACTA2-positive pericytes exclusively expressed GFP, demonstrating that nestin-GFP-positive pericytes derived from tissue-resident cells and not circulating (BM-derived) MSCs stabilize angiogenic vessels in tumours grown in those mice. In combination with intensive immunofluorescence analysis, these results strongly confirmed the hypothesis that nestin-GFP-positive MSCs are apparently involved directly in vascular remodelling processes in terms of vascular

stabilization, serving as a major source for pericytes and SMC. Thus, vascular wall-resident MSCs have to be considered in future strategies for anti-angiogenic tumour therapy. According to this idea, nestin expression of human colorectal adenocarcinoma metastases under clinical treatment with bevacizumab showed a prominent stabilization of tumour vessels by increased integration of nestin-positive pericytes and/or SMC into the vessel wall [81]. Mature vessels from the tumour's surrounding area or healthy tissue, by contrast, down-regulated nestin expression. Nestin expression had already been considered to be specific for developing vascular smooth muscle cells (VSMC), whereas differentiated, postmitotic VSMC were negative for nestin [88]. Conclusively, nestin-targeted therapy may suppress tumour proliferation via inhibition of neovascularization and vessel stabilization in numerous malignancies, including colorectal cancer and melanomas. Nestin, an intermediate filament protein, is reportedly expressed in repair processes, various neoplasms, and proliferating vascular endothelial cells [89, 90]. It was recently reported to be expressed in proliferating endothelial progenitor cells, but not in mature endothelial cells. Tumour endothelium-specific expression is thought to depend on the first intron of the nestin gene, whereas neural stem cell-specific and thus MSC-specific expression is usually regulated by the second intron [90]. Therefore, expression of nestin was described to be relatively limited in proliferating vascular endothelial cells and EPCs. Using another but similarly constructed nestin-GFP plasmid generated nestin-GFP transgenic mouse, nestin-positive pericytes have been identified as the progenitors of all Leydig cell phenotypes, indicating that vascular cell types, acting like adult stem cells, play a critical role in organ formation [91]. Thus, these findings confirm the idea that addressing pericytes, in particular by nestin-targeted therapy, may be suitable to selectively address newly formed and partially stabilized tumour blood vessels.

From the literature, it appears to still be controversial whether and to what extent BM-derived vascular progenitor cells or tissue-resident stem and progenitor cells contribute to neovascularization processes. BM-derived endothelial progenitor cells (BM-EPCs) have been shown to represent an alternative source of endothelial cells for adult neovascularization in the process defined as postnatal vasculogenesis [92, 93]. Thus, BM-EPCs might constitute a new and promising target for pro- or anti-angiogenic treatment strategies [94]. However, there is extensive variation about their contribution to tumour neovascularization of primary tumours, and the respective values range from 50 % incorporated BM-EPCs to undetectable numbers, demonstrating that the exact role of these cells in postnatal vasculogenesis is not quite clear [95-97]. These contradictory results may be due to the methodological difficulties in distinguishing BM-derived cells from intimately associated cells [94]. Furthermore, the effects of MSCs on tumour growth are still controversial. Interactions between MSCs and tumour cells might play an important role in tumour growth [98, 99]. Herein, MSCs have been shown to transmit their tumour-promoting activity via a paracrine mechanism of action: conditioned media derived from cultured BM-MSCs induced the expression of VEGF in tumour cells as well as the activation RhoA-GTPase and ERK1/2 [100]. Furthermore, BM-derived MSCs (also called mesenchymal stromal cells) have been reported to migrate to the site of tumour progression and to subsequently differentiate into carcinoma-associated fibroblast (CAF)-like cells, thereby representing tumour-promoting stromal cells. As CAFs express platelet-derived growth factor receptor

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(PDGFR) at a high level, a blockade of PDGF signalling pathways by imatinib treatment influenced the interaction between BM-derived MSCs and tumour cells in the tumour microenvironment and, hence, inhibited the progressive growth of colon cancer [101].

In general, considerable evidence is accumulating for the involvement of tissue-resident and in particular vessel-associated MPSCs in regenerative and pathological adult neovascularization [43, 102, 103]. In vitro experiments further suggested that proliferative SMCs are derived from the differentiation of multipotent vascular stem cell (MVSC) of the blood vessel wall instead of the de-differentiation of mature SMCs [104]. MVSCs-expressed markers including Sox17, Sox10 and S100β were cloneable, had telomerase activity, and differentiated into neural cells and mesenchymal stem cell (MSC)-like cells that subsequently differentiated into SMCs. In vivo experiments further demonstrated that MVSCs, rather than mature SMCs, repopulate the tunica media and form neointima after endothelial denudation injury [104]. Whether MVSCs were derived from the de-differentiation of mature SMCs was determined by lineage tracing using SM-MHC as a marker in SM-MHC-Cre/LoxP-enhanced green fluorescence protein (EGFP) mice [105, 106]. These studies support the hypothesis that vascular multipotent stem cells of a mesenchymal nature were activated and generated SMC by differentiation instead of a possible SMC de-differentiation of the vascular wall. We may conclude that, in addition to their above-described role in tumour vascularization, the aberrant activation and differentiation of vascular wall-resident multipotent stem cells may contribute the development of vascular diseases. These findings may have a transformative impact on vascular biology, vascular diseases and remodelling, and may lead to new therapies by using VW-MPSCs as a therapeutic target.

4. Regulation of differentiation of vascular wall-resident multipotent stem cells into smooth muscle cells

Epigenetic regulation was shown to play a crucial role in SMC differentiation [107]. High levels of histone modifications were found in promoters of SMC-specific genes as compared to undifferentiated embryonic stem cells [108, 109]. Of the epigenetic regulation mechanisms, histone acetylation, which is adjusted through acetyltransferases (HATs) and histone deacetylases (HDACs), primarily promotes the expression of target genes [110]. However, whether the differentiation of MSCs to SMCs was affected by such histone modifications remains unresolved. HDACs, however, can arrest stem cell proliferation and induce cell differentiation and apoptosis [111]. A histone deacetylase inhibitor (sodium butyrate) was further found to effectively promote rat BM-MSC differentiation into SMCs; a strategy that could potentially be applied in clinical tissue engineering and cell transplantation, for example for the treatment of bladder function disorders such as stress urinary incontinence [112, 113].

In order to identify molecular mechanisms governing the differentiation of the vascular wall-resident MPSCs into SMCs, cDNA microarray analyses on MPSCs isolated from human internal thoracic artery fragments in comparison to mature SMC of human aorta were performed (unpublished data). Among several genes being differentially expressed in VW-

MPSCs, the HOX genes HOXB7, HOXC6 and HOXC8 were found to be expressed in VW-MPSCs at a clearly higher level than in mature hAoSMC [60]. The HOX genes are a family of regulatory transcription factors that control the activity of other functionally related genes in the course of individual development, and are expressed variously in the adult organism [114]. Because of their central role in the development of body parts, limbs and organs, mutation of these genes can cause serious changes in body parts at points in the body that they do not physiologically occur, such as the conversion of complete limbs. In humans, so far, HOX-39 transcription factors have been identified in the four separate clusters (HOXA-D) that are located on four different chromosomes. Together with accessory factors, HOX proteins bind to specific DNA sequences in order to activate or repress genes [115]. HOX genes are thought to act as micromanagers orchestrating cell differentiation after embryonic development in many different cell types and developmental pathways [116]. In the adult, it is already known that colony-forming unit-fibroblasts (CFU-F) derived from different organs have characteristic HOX expression signatures that are heterogeneous but highly specific for their anatomical origin [117]. The topographic specificity of HOX code is maintained during differentiation, which indeed suggests that the pattern of expression is an intrinsic property of MSCs. Furthermore, stem and progenitor cells from mesodermal tissues have HOX-specific gene expression profiles. This so-called biological fingerprint can be used to differentiate functionally different MSC populations from bone marrow and umbilical cord blood [118]. Thus, HOX proteins have a role in specifying the cellular identity of MSC. A differential analysis of 39 HOX genes in vascular wall-resident MPSCs compared to terminally differentiated endothelial cells, SMC and less differentiated (pluripotent) embryonic stem cells showed that HOX family members HOXB7, HOXC6 and HOXC8 are overexpressed in the vessel-resident MPSCs. This suggests that these HOX genes are involved in the development and differentiation of the VW-MPSCs [60]. To gain further insights into the molecular role of these HOX genes for VW-MPSC differentiation as well as to identify potential downstream regulated genes of HOXB7, HOXC6 and HOXC8 activity, VW-MPSCs were transfected with HOXB7, HOXC6 and HOXC8-specific siRNAs both individually and in defined combinations using non-specific siRNAs as controls. Interestingly, silencing these HOX genes in VW-MPSCs significantly reduced their sprouting capacity and increased expression of the SMC differentiation and maturation markers transgelin (TAGLN) and calponin (CNN1), and the histone gene histone H1. Furthermore, the methylation pattern of the TAGLN promoter was altered, which clearly indicates a differentiation of VW-MPSCs to a more mature SMC phenotype. A restricted expression of HOX genes, in particular HOXB7, had already been reported in the 1990s to distinguish foetal from adult human SMC, whereby HOXB7 was expressed at markedly higher levels in embryonic vascular SMC as compared to mature SMC of adult vessels [119]. These data suggest that HOXB7 initiates a differentiation from multipotent cell type towards SMC, but stops the further differentiation of these cells into mature SMC. Further striking evidence is that H1 is also involved in the regulation of VW-MPSC differentiation into SMC [60]. H1 expression in VW-MPSCs is significantly enhanced upon differentiation towards SMC, as shown after gene silencing for HOXB7, HOXC6 and HOXC8, respectively. In general, H1 function can alter the chromatin structure and serves as both a positive

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and negative regulator of transcription, depending on the gene. H1 can further influence DNA methylation and regulate specific gene expression [120-122]. We may conclude that the interaction of H1 and HOXB7 might be a more specific mechanism regulating gene expression and differentiation of VW-MPSCs to SMCs and then to mature SMCs in physiological remodelling processes of the vessel wall and vascular diseases. Indeed, in human atherosclerotic lesions, where mature SMCs revert to a more immature and less contractile phenotype, HOXB7 mRNA was detected at a higher level than in normal artery wall [123]. An even closer relationship seems to exist between VW-MPSCs and mature SMCs. SMC differentiation is accompanied by enhanced ACTA2, TAGLN and CNN1 expression. TAGLN is expressed exclusively in smooth muscle-containing tissues of adult mammals, and is one of the earliest markers of differentiating SMCs [124]. While the expression of these markers is a common feature of SMC regardless of their anatomical position, it has been shown that even SMCs of different parts of adult arteries, e.g., aortic arch, abdominal aorta and femoral artery, exhibit different codes of HOX gene expression, indicating the close relation between HOX code and the anatomical positional identity of SMC in each part of the blood vessels [125].

Further candidate factors were reported to be important for MSC differentiation to SMC. The most prominent one is the morphogenetic transforming growth factor-beta (TGFb) [126]. TGFb stimulation alone is sufficient for the induction of a rapid SMC differentiation of MPSC and MSC-like cells [46, 127, 128]. Isolated VW-MPSCs exposed to exogenous TGFβ1 during culturing exhibited alterations in the gene expression profile in the form of significantly increased expression of the SMC markers TAGLN, hyaluronan and proteoglycan binding link protein (HAPLN), and thrombospondin 1 (THSP1) [46]. In embryonic stem cell-derived MSCs (hES-MCs), TGF-β-treatment resulted in SMC differentiation in a dose- and time-dependent manner as demonstrated by the expression of SMC-specific genes ACTA2, CNN1, and smooth muscle myosin heavy chain (SM-MHC) [127]. Mechanistically, TGFb-induced differentiation was Smad- and serum response factor/myocardin-dependent. Furthermore, the treatment of adipose tissue-derived MSCs (hASCs) with TGFb dramatically increased the contraction of a collagen-gel lattice and the expression levels of SMC-specific genes including ACTA2, CNN1, SM-MHC, smoothelin-B, myocardin and h-caldesmon, as well as causing an increased expression of vascular SMC-like ion channels, indicating differentiation of hASCs into contractile vascular SMCs [128]. Beside the direct action of growth and differentiation factors, either by direct, exogenous application to cultured MPSC and MSC-like cells, or by stimulation of vascular MPSC in their native niche (e.g., by tumour secretion), other factors were described as decisive for the SMC differentiation of vascular multipotent stem cells. The differential expression of these cell-type-specific factors seems to act more indirectly, and to prime the cell somehow to differentiate along the SMC lineage. The basic molecular mechanism behind these cell-type-specific factors remains elusive at present. A prominent EphA3 expression in endometrial spiral arterioles and surrounding stroma, but not in other human tissues, suggests EphA3 as a unique marker of perivascular MSCs that are implicated in rapid neovascularization and vascular remodelling [129]. This selective EphA3 expression was further observed in actively growing rather than established blood vessels in the vascular microenvironment of solid tumours. In addition, a strong expression of CD146 within a BM-MSC subpopulation

was associated with a commitment to a vascular smooth muscle cell lineage characterized by a strong up-regulation of calponin-1 and SM22α expression and an ability to contract the collagen matrix [130].

5. Concluding remarks

Angiogenesis and vasculogenesis are central events in tissue development and repair. Initially, sprouting endothelial cells form immature blood vessels that lack coverage by pericytes and other mural cells. Subsequently vascular remodelling takes place, in which association with mural cells (pericytes and SMC) stabilizes these immature vessels. Vascular remodelling is a dynamic and strictly regulated process, which is active in a variety of physiological processes, such as vessel growth, angiogenesis and wound healing. An ordered remodelling seems to be critical for proper vascular development and maintenance and is an absolute prerequisite to preserve the sensitive relationship between resilience and stability of the vessel wall. However, remodelling is also initiated during pathological processes, such as atherosclerosis, ischaemia, congenital vascular lesions, vasculotoxic therapies and tumour growth.

Organ-specific multipotent stem cell types are associated with the vessel wall, in particular within the so-called "vasculogenic zone" of the vascular adventitia. These findings together with the stem cell-supporting functions of endothelial cells suggest that the vascular wall provides niches for different somatic stem cell types within the sub-endothelial space and the vascular adventitia. It conformity with the niche function of the adventitial vasculogenic zone, the presence of Sca-1+ smooth muscle cell progenitors has been shown within this zone [131]. Furthermore, it was reported that a subset of CD34+ cells within the vascular adventitia has the capacity to differentiate into pericytes [132]. More recently, CD44(+)CD90(+)CD73(+)CD34(-)CD45(-) cells were identified within the adult human arterial adventitia, which were termed vascular wall-resident multipotent stem cells (VW-MPSCs) and were capable of differentiating into vascular SMC and pericytes under in vitro and in vivo conditions [46]. These cells reside predominantly in the vasculogenic zone of adult human blood vessels and contribute to maturation of newly formed vessels.

In general, tissue-specific stem cells differentiate mainly to the type of tissue from which they derive, indicating that there might be a certain code ("priming") within the cells determined by the tissue of origin. Furthermore, due to their anatomical localization it is believed the vessel-resident stem and progenitor cells are available as a first point of contact for the secreted factors from tumour cells (Figure 5). Without mobilization from the niche, VW-MPSCs express HOXB7, HOXC6 and HOXC8 at higher levels as compared to SMCs. These HOX genes suppress the expression of TAGLN and CNN1 in VW-MPSCs, essential factors of early SMC differentiation. This mechanism probably accounts for keeping the VW-MPSCs quiescent in the adventitial niche. In contrast, silencing of HOX genes alter the CpG methylation of TAGLN promotor resulted in increased TAGLN expression which induced VW-MPSC differentiation into SMC/pericytes [60]. Thus, as discussed here in detail, VW-MPSCs are directly involved in vascular remodelling processes as these cells represent the major source of pericytes and SMC during angiogenesis and vascular stabilization processes under physiological and pathological

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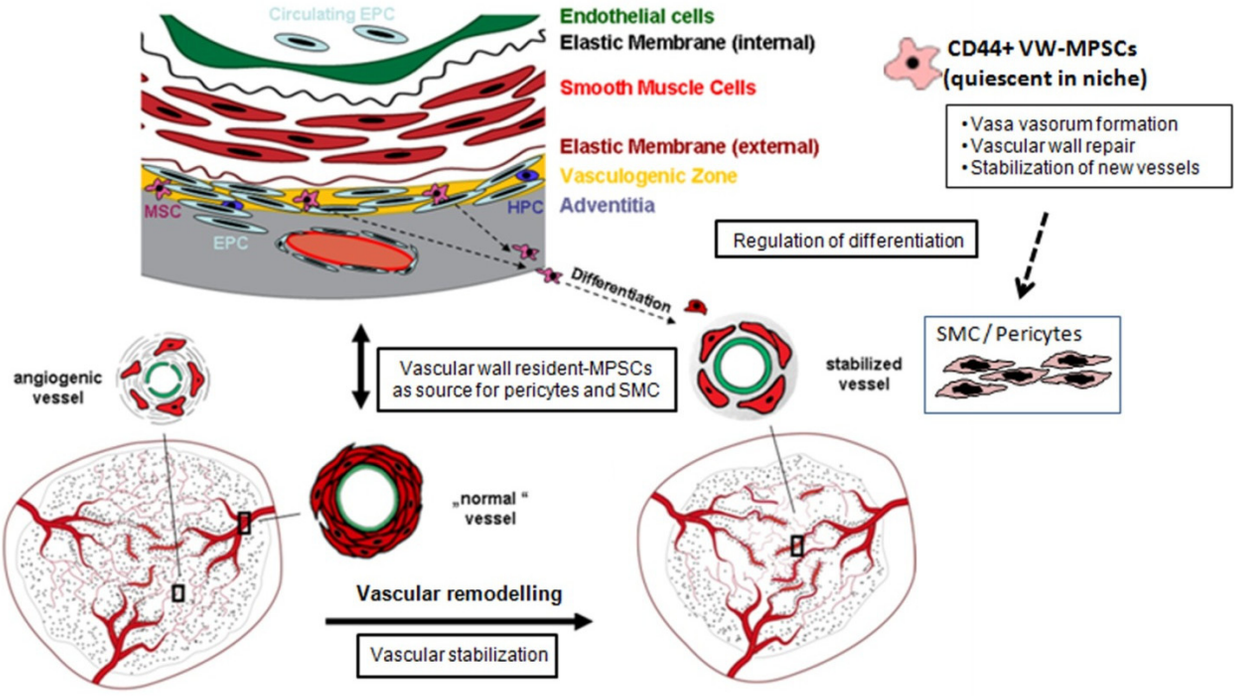


Figure 5. Vascular wall-resident multipotent stem cells (VW-MPSCs). VW-MPSC mobilization and differentiation into SMC may also be induced by signals released from, e.g., tumours, inflammation and hypoxia in tissue areas around blood vessels contributing to morphogenesis of the new vessel wall. It is hypothesized that VW-MPSCs are the "first-line" cells which were mobilized from their niche towards the tumour and activated to differentiate into pericytes and SMCs, which in turn stabilize angiogenic blood vessels. The molecular analysis of these stabilizing wall cells could yield new strategies for single-target genes, which could reduce the rate of drug-resistant tumours. Without mobilization from the niche VW-MPSCs express specific transcription factors at high levels. These genes suppress the expression of SMC-specific genes in VW-MPSCs. This mechanism probably accounts for keeping the VW-MPSCs quiescent in the adventitial niche. Tissue-resident MPSC and in particular VW-MPSCs, rather than circulating (BM-derived) multipotent stem cells, represent the major source of pericytes and SMCs during tumour vascularization. These findings on the origin of vascular-stabilizing pericytes and their function in tumour vascularization and remodelling provide a further basis for the design of novel strategies to improve anti-angiogenic therapies.

conditions. Therefore, these cells may be a promising target for counteracting vascular remodelling and related anti-angiogenic drug resistance.

In future investigations, a detailed molecular analysis of vascular wall-resident multipotent stem cells and of their differentiation into pericytes in response to tumour-secreted factors may be decisive to gain a better understanding of MPSC biology and differentiation. Particularly for cancer therapy, there is an urgent need to identify signalling molecules that are selectively regulated during the process of new vessel formation and/or subsequent vascular stabilization. Targeting of such molecules might also help to minimize anti-angiogenic drug resistance due to vascular stabilization. These investigations will provide basic knowledge for the design of innovative therapeutic strategies that target those vascular remodelling processes during cancer treatment that are associated with worse prognosis, for example, the generation of drug-resistant tumours.

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References

[1] Nagy JA, Benjamin L, Zeng H, Dvorak AM, Dvorak HF. Vascular permeability, vascular hyperpermeability and angiogenesis. Angiogenesis. 2008;11(2):109-19. Epub 2008/02/23.

[2] Carmeliet P. Angiogenesis in health and disease. Nature medicine. 2003;9(6):653-60. Epub 2003/06/05.

[3] Ferrara N, Kerbel RS. Angiogenesis as a therapeutic target. Nature. 2005;438(7070): 967-74. Epub 2005/12/16.

[4] Jain RK. Normalizing tumor vasculature with anti-angiogenic therapy: a new paradigm for combination therapy. Nature medicine. 2001;7(9):987-9. Epub 2001/09/05.

[5] Zengin E, Chalajour F, Gehling UM, Ito WD, Treede H, Lauke H, et al. Vascular wall resident progenitor cells: a source for postnatal vasculogenesis. Development. 2006;133(8):1543-51. Epub 2006/03/10.

[6] Gupta MK, Qin RY. Mechanism and its regulation of tumor-induced angiogenesis. World journal of gastroenterology : WJG. 2003;9(6):1144-55. Epub 2003/06/12.

[7] Benjamin LE, Golijanin D, Itin A, Pode D, Keshet E. Selective ablation of immature blood vessels in established human tumors follows vascular endothelial growth factor withdrawal. The Journal of clinical investigation. 1999;103(2):159-65. Epub 1999/01/23.

[8] Gee MS, Procopio WN, Makonnen S, Feldman MD, Yeilding NM, Lee WM. Tumor vessel development and maturation impose limits on the effectiveness of anti-vascular therapy. The American journal of pathology. 2003;162(1):183-93. Epub 2003/01/01.

[9] Tilki D, Kilic N, Sevinc S, Zywietz F, Stief CG, Ergun S. Zone-specific remodeling of tumor blood vessels affects tumor growth. Cancer. 2007;110(10):2347-62. Epub 2007/09/13.

[10] Jain RK, Finn AV, Kolodgie FD, Gold HK, Virmani R. Antiangiogenic therapy for normalization of atherosclerotic plaque vasculature: a potential strategy for plaque stabilization. Nature clinical practice Cardiovascular medicine. 2007;4(9):491-502. Epub 2007/08/23.

[11] Weisshardt P, Trarbach T, Durig J, Paul A, Reis H, Tilki D, et al. Tumor vessel stabilization and remodeling by anti-angiogenic therapy with bevacizumab. Histochemistry and cell biology. 2012;137(3):391-401. Epub 2011/12/24.

[12] Murakami M. Signaling required for blood vessel maintenance: molecular basis and pathological manifestations. International journal of vascular medicine. 2012;2012:293641. Epub 2011/12/22.

[13] Adams RH, Alitalo K. Molecular regulation of angiogenesis and lymphangiogenesis. Nature reviews Molecular cell biology. 2007;8(6):464-78. Epub 2007/05/25.

[14] Asahara T, Murohara T, Sullivan A, Silver M, van der Zee R, Li T, et al. Isolation of putative progenitor endothelial cells for angiogenesis. Science. 1997;275(5302):964-7. Epub 1997/02/14.

[15] Malik AK, Baldwin ME, Peale F, Fuh G, Liang WC, Lowman H, et al. Redundant roles of VEGF-B and PlGF during selective VEGF-A blockade in mice. Blood. 2006;107(2):550-7. Epub 2005/09/29.

[16] Gerber HP, Ferrara N. Pharmacology and pharmacodynamics of bevacizumab as monotherapy or in combination with cytotoxic therapy in preclinical studies. Cancer research. 2005;65(3):671-80. Epub 2005/02/12.

[17] Gaengel K, Genove G, Armulik A, Betsholtz C. Endothelial-mural cell signaling in vascular development and angiogenesis. Arteriosclerosis, thrombosis, and vascular biology. 2009;29(5):630-8. Epub 2009/01/24.

[18] Carmeliet P, Jain RK. Molecular mechanisms and clinical applications of angiogenesis. Nature. 2011;473(7347):298-307. Epub 2011/05/20.

[19] Giordano FJ, Johnson RS. Angiogenesis: the role of the microenvironment in flipping the switch. Current opinion in genetics & development. 2001;11(1):35-40. Epub 2001/02/13.

[20] Furuya M, Yonemitsu Y, Aoki I. III. Angiogenesis: complexity of tumor vasculature and microenvironment. Current pharmaceutical design. 2009;15(16):1854-67. Epub 2009/06/13.

[21] Ergun S, Kilic N, Wurmbach JH, Ebrahimnejad A, Fernando M, Sevinc S, et al. Endostatin inhibits angiogenesis by stabilization of newly formed endothelial tubes. Angiogenesis. 2001;4(3):193-206. Epub 2002/03/26.

[22] Ergun S, Tilki D, Oliveira-Ferrer L, Schuch G, Kilic N. Significance of vascular stabilization for tumor growth and metastasis. Cancer letters. 2006;238(2):180-7. Epub 2005/08/09.

[23] Goel S, Duda DG, Xu L, Munn LL, Boucher Y, Fukumura D, et al. Normalization of the vasculature for treatment of cancer and other diseases. Physiological reviews. 2011;91(3):1071-121. Epub 2011/07/12.

[24] Jain RK. Antiangiogenic therapy for cancer: current and emerging concepts. Oncology (Williston Park). 2005;19(4 Suppl 3):7-16. Epub 2005/06/07.

[25] Sun J, Wang DA, Jain RK, Carie A, Paquette S, Ennis E, et al. Inhibiting angiogenesis and tumorigenesis by a synthetic molecule that blocks binding of both VEGF and PDGF to their receptors. Oncogene. 2005;24(29):4701-9. Epub 2005/05/18.

[26] Jain RK. Normalization of tumor vasculature: an emerging concept in antiangiogenic therapy. Science. 2005;307(5706):58-62. Epub 2005/01/08.

[27] Gasparini G, Longo R, Toi M, Ferrara N. Angiogenic inhibitors: a new therapeutic strategy in oncology. Nature clinical practice Oncology. 2005;2(11):562-77. Epub 2005/11/05.

[28] Ferrara N, Hillan KJ, Novotny W. Bevacizumab (Avastin), a humanized anti-VEGF monoclonal antibody for cancer therapy. Biochemical and biophysical research communications. 2005;333(2):328-35. Epub 2005/06/18.

[29] Ferrara N. Napoleone Ferrara discusses Avastin and the future of anti-angiogenesis therapy. Interview by Ulrike Knies-Bamforth and Christopher Watson. Drug discovery today. 2005;10(8):539-41. Epub 2005/04/20.

[30] Xue Y, Religa P, Cao R, Hansen AJ, Lucchini F, Jones B, et al. Anti-VEGF agents confer survival advantages to tumor-bearing mice by improving cancer-associated systemic syndrome. Proceedings of the National Academy of Sciences of the United States of America. 2008;105(47):18513-8. Epub 2008/11/20.

[31] Zhuang HQ, Yuan ZY. Process in the mechanisms of endostatin combined with radiotherapy. Cancer letters. 2009;282(1):9-13. Epub 2009/01/13.

[32] Helfrich I, Scheffrahn I, Bartling S, Weis J, von Felbert V, Middleton M, et al. Resistance to antiangiogenic therapy is directed by vascular phenotype, vessel stabilization, and maturation in malignant melanoma. The Journal of experimental medicine. 2010;207(3):491-503. Epub 2010/03/03.

[33] Puthillath A, Patel A, Fakih MG. Targeted therapies in the management of colorectal carcinoma: role of bevacizumab. OncoTargets and therapy. 2009;2:1-15. Epub 2009/01/01.

[34] Erber R, Thurnher A, Katsen AD, Groth G, Kerger H, Hammes HP, et al. Combined inhibition of VEGF and PDGF signaling enforces tumor vessel regression by interfering with pericyte-mediated endothelial cell survival mechanisms. FASEB journal : official publication of the Federation of American Societies for Experimental Biology. 2004;18(2):338-40. Epub 2003/12/06.

[35] Greenberg JI, Shields DJ, Barillas SG, Acevedo LM, Murphy E, Huang J, et al. A role for VEGF as a negative regulator of pericyte function and vessel maturation. Nature. 2008;456(7223):809-13. Epub 2008/11/11.

[36] Lin MI, Sessa WC. Antiangiogenic therapy: creating a unique "window" of opportunity. Cancer cell. 2004;6(6):529-31. Epub 2004/12/21.

[37] Ribatti D, Nico B, Crivellato E. The role of pericytes in angiogenesis. The International journal of developmental biology. 2011;55(3):261-8. Epub 2011/06/29.

[38] O'Reilly MS. Radiation combined with antiangiogenic and antivascular agents. Seminars in radiation oncology. 2006;16(1):45-50. Epub 2005/12/28.

[39] Timke C, Zieher H, Roth A, Hauser K, Lipson KE, Weber KJ, et al. Combination of vascular endothelial growth factor receptor/platelet-derived growth factor receptor inhibition markedly improves radiation tumor therapy. Clinical cancer research : an official journal of the American Association for Cancer Research. 2008;14(7):2210-9. Epub 2008/04/03.

[40] Digklia A, Voutsadakis IA. Combinations of vascular endothelial growth factor pathway inhibitors with metronomic chemotherapy: Rational and current status. World journal of experimental medicine. 2014;4(4):58-67. Epub 2014/11/22.

[41] Pasquinelli G, Tazzari PL, Vaselli C, Foroni L, Buzzi M, Storci G, et al. Thoracic aortas from multiorgan donors are suitable for obtaining resident angiogenic mesenchymal stromal cells. Stem Cells. 2007;25(7):1627-34. Epub 2007/04/21.

[42] Invernici G, Emanueli C, Madeddu P, Cristini S, Gadau S, Benetti A, et al. Human fetal aorta contains vascular progenitor cells capable of inducing vasculogenesis, angiogenesis, and myogenesis in vitro and in a murine model of peripheral ischemia. The American journal of pathology. 2007;170(6):1879-92. Epub 2007/05/26.

[43] Ergun S, Tilki D, Klein D. Vascular wall as a reservoir for different types of stem and progenitor cells. Antioxidants & redox signaling. 2011;15(4):981-95. Epub 2010/08/18.

[44] Majesky MW, Dong XR, Hoglund V, Daum G, Mahoney WM, Jr. The adventitia: a progenitor cell niche for the vessel wall. Cells, tissues, organs. 2012;195(1-2):73-81. Epub 2011/10/19.

[45] Chen Y, Wong MM, Campagnolo P, Simpson R, Winkler B, Margariti A, et al. Adventitial stem cells in vein grafts display multilineage potential that contributes to neointimal formation. Arteriosclerosis, thrombosis, and vascular biology. 2013;33(8): 1844-51. Epub 2013/06/08.

[46] Klein D, Weisshardt P, Kleff V, Jastrow H, Jakob HG, Ergun S. Vascular wall-resident CD44+ multipotent stem cells give rise to pericytes and smooth muscle cells and contribute to new vessel maturation. PloS one. 2011;6(5):e20540. Epub 2011/06/04.

[47] Psaltis PJ, Harbuzariu A, Delacroix S, Witt TA, Holroyd EW, Spoon DB, et al. Identification of a monocyte-predisposed hierarchy of hematopoietic progenitor cells in the adventitia of postnatal murine aorta. Circulation. 2012;125(4):592-603. Epub 2011/12/29.

[48] Stenmark KR, Davie N, Frid M, Gerasimovskaya E, Das M. Role of the adventitia in pulmonary vascular remodeling. Physiology (Bethesda). 2006;21:134-45. Epub 2006/03/28.

[49] Hoshino A, Chiba H, Nagai K, Ishii G, Ochiai A. Human vascular adventitial fibroblasts contain mesenchymal stem/progenitor cells. Biochemical and biophysical research communications. 2008;368(2):305-10. Epub 2008/01/31.

[50] Ruan J, Luo M, Wang C, Fan L, Yang SN, Cardenas M, et al. Imatinib disrupts lymphoma angiogenesis by targeting vascular pericytes. Blood. 2013;121(26):5192-202. Epub 2013/05/02.

[51] Kano MR, Komuta Y, Iwata C, Oka M, Shirai YT, Morishita Y, et al. Comparison of the effects of the kinase inhibitors imatinib, sorafenib, and transforming growth factor-beta receptor inhibitor on extravasation of nanoparticles from neovasculature. Cancer science. 2009;100(1):173-80. Epub 2008/11/29.

[52] Song S, Ewald AJ, Stallcup W, Werb Z, Bergers G. PDGFRbeta+ perivascular progenitor cells in tumours regulate pericyte differentiation and vascular survival. Nature cell biology. 2005;7(9):870-9. Epub 2005/08/23.

[53] Nicosia RF, Villaschi S. Autoregulation of angiogenesis by cells of the vessel wall. International review of cytology. 1999;185:1-43. Epub 1998/09/29.

[54] Nehls V, Denzer K, Drenckhahn D. Pericyte involvement in capillary sprouting during angiogenesis in situ. Cell and tissue research. 1992;270(3):469-74. Epub 1992/12/01.

[55] Bergers G, Benjamin LE. Tumorigenesis and the angiogenic switch. Nature reviews Cancer. 2003;3(6):401-10. Epub 2003/06/05.

[56] Stenmark KR, Yeager ME, El Kasmi KC, Nozik-Grayck E, Gerasimovskaya EV, Li M, et al. The adventitia: essential regulator of vascular wall structure and function. Annual review of physiology. 2013;75:23-47. Epub 2012/12/12.

[57] 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):1530-9. Epub 2011/06/17.

[58] Hu Y, Xu Q. Adventitial biology: differentiation and function. Arteriosclerosis, thrombosis, and vascular biology. 2011;31(7):1523-9. Epub 2011/06/17.

[59] Torsney E, Xu Q. Resident vascular progenitor cells. Journal of molecular and cellular cardiology. 2011;50(2):304-11. Epub 2010/09/21.

[60] Klein D, Benchellal M, Kleff V, Jakob HG, Ergun S. Hox genes are involved in vascular wall-resident multipotent stem cell differentiation into smooth muscle cells. Scientific reports. 2013;3:2178. Epub 2013/10/23.

[61] Tilki D, Hohn HP, Ergun B, Rafii S, Ergun S. Emerging biology of vascular wall progenitor cells in health and disease. Trends in molecular medicine. 2009;15(11):501-9. Epub 2009/10/16.

[62] Gronthos S, Zannettino AC, Hay SJ, Shi S, Graves SE, Kortesidis A, et al. Molecular and cellular characterisation of highly purified stromal stem cells derived from human bone marrow. Journal of cell science. 2003;116(Pt 9):1827-35. Epub 2003/04/01.

[63] Tropel P, Noel D, Platet N, Legrand P, Benabid AL, Berger F. Isolation and characterisation of mesenchymal stem cells from adult mouse bone marrow. Experimental cell research. 2004;295(2):395-406. Epub 2004/04/20.

[64] Augello A, Kurth TB, De Bari C. Mesenchymal stem cells: a perspective from in vitro cultures to in vivo migration and niches. European cells & materials. 2010;20:121-33. Epub 2011/01/21.

[65] Secunda R, Vennila R, Mohanashankar AM, Rajasundari M, Jeswanth S, Surendran R. Isolation, expansion and characterisation of mesenchymal stem cells from human bone marrow, adipose tissue, umbilical cord blood and matrix: a comparative study. Cytotechnology. 2014. Epub 2014/05/07.

[66] Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, et al. Multilineage potential of adult human mesenchymal stem cells. Science. 1999;284(5411): 143-7. Epub 1999/04/02.

[67] Pikula M, Marek-Trzonkowska N, Wardowska A, Renkielska A, Trzonkowski P. Adipose tissue-derived stem cells in clinical applications. Expert opinion on biological therapy. 2013;13(10):1357-70. Epub 2013/08/08.

[68] Vellasamy S, Sandrasaigaran P, Vidyadaran S, George E, Ramasamy R. Isolation and characterisation of mesenchymal stem cells derived from human placenta tissue. World journal of stem cells. 2012;4(6):53-61. Epub 2012/09/21.

[69] Hayward CJ, Fradette J, Galbraith T, Remy M, Guignard R, Gauvin R, et al. Harvesting the potential of the human umbilical cord: isolation and characterisation of four

cell types for tissue engineering applications. Cells, tissues, organs. 2013;197(1):37-54. Epub 2012/09/12.

[70] Gregoire-Gauthier J, Selleri S, Fontaine F, Dieng MM, Patey N, Despars G, et al. Therapeutic efficacy of cord blood-derived mesenchymal stromal cells for the prevention of acute graft-versus-host disease in a xenogenic mouse model. Stem cells and development. 2012;21(10):1616-26. Epub 2011/09/14.

[71] Gerlach JC, Over P, Turner ME, Thompson RL, Foka HG, Chen WC, et al. Perivascular mesenchymal progenitors in human fetal and adult liver. Stem cells and development. 2012;21(18):3258-69. Epub 2012/08/31.

[72] da Silva Meirelles L, Caplan AI, Nardi NB. In search of the in vivo identity of mesenchymal stem cells. Stem Cells. 2008;26(9):2287-99. Epub 2008/06/21.

[73] Crisan M, Yap S, Casteilla L, Chen CW, Corselli M, Park TS, et al. A perivascular origin for mesenchymal stem cells in multiple human organs. Cell stem cell. 2008;3(3): 301-13. Epub 2008/09/13.

[74] Lin CS, Lue TF. Defining vascular stem cells. Stem cells and development. 2013;22(7): 1018-26. Epub 2013/01/22.

[75] Majesky MW, Dong XR, Regan JN, Hoglund VJ. Vascular smooth muscle progenitor cells: building and repairing blood vessels. Circulation research. 2011;108(3):365-77. Epub 2011/02/05.

[76] Lin G, Garcia M, Ning H, Banie L, Guo YL, Lue TF, et al. Defining stem and progenitor cells within adipose tissue. Stem cells and development. 2008;17(6):1053-63. Epub 2008/07/04.

[77] Matsuda Y, Hagio M, Ishiwata T. Nestin: a novel angiogenesis marker and possible target for tumor angiogenesis. World journal of gastroenterology : WJG. 2013;19(1): 42-8. Epub 2013/01/18.

[78] Ribatti D. Vascular normalization: a real benefit? Cancer chemotherapy and pharmacology. 2011;68(2):275-8. Epub 2011/06/04.

[79] Huang Y, Goel S, Duda DG, Fukumura D, Jain RK. Vascular normalization as an emerging strategy to enhance cancer immunotherapy. Cancer research. 2013. Epub 2013/02/27.

[80] Lee S. What tumor vessels can tell us. Pigment cell & melanoma research. 2010;23(3): 309-11. Epub 2010/04/21.

[81] Klein D, Meissner N, Kleff V, Jastrow H, Yamaguchi M, Ergun S, et al. Nestin(+) tissue-resident multipotent stem cells contribute to tumor progression by differentiating into pericytes and smooth muscle cells resulting in blood vessel remodeling. Frontiers in oncology. 2014;4:169. Epub 2014/07/16.

[82] Yamaguchi M. Analysis of neurogenesis using transgenic mice expressing GFP with nestin gene regulatory regions. Chemical senses. 2005;30 Suppl 1:i117-8. Epub 2005/03/02.

[83] Matsumura S, Takagi K, Okuda-Ashitaka E, Lu J, Naritsuka H, Yamaguchi M, et al. Characterization of nestin expression in the spinal cord of GFP transgenic mice after peripheral nerve injury. Neuroscience. 2010;170(3):942-53. Epub 2010/08/03.

[84] Lin RZ, Moreno-Luna R, Zhou B, Pu WT, Melero-Martin JM. Equal modulation of endothelial cell function by four distinct tissue-specific mesenchymal stem cells. Angiogenesis. 2012;15(3):443-55. Epub 2012/04/25.

[85] Au P, Tam J, Fukumura D, Jain RK. Bone marrow-derived mesenchymal stem cells facilitate engineering of long-lasting functional vasculature. Blood. 2008;111(9): 4551-8. Epub 2008/02/08.

[86] Krampera M, Galipeau J, Shi Y, Tarte K, Sensebe L. Immunological characterization of multipotent mesenchymal stromal cells-The International Society for Cellular Therapy (ISCT) working proposal. Cytotherapy. 2013. Epub 2013/04/23.

[87] Mendez-Ferrer S, Michurina TV, Ferraro F, Mazloom AR, Macarthur BD, Lira SA, et al. Mesenchymal and haematopoietic stem cells form a unique bone marrow niche. Nature. 2010;466(7308):829-34. Epub 2010/08/13.

[88] Oikawa H, Hayashi K, Maesawa C, Masuda T, Sobue K. Expression profiles of nestin in vascular smooth muscle cells in vivo and in vitro. Experimental cell research. 2010;316(6):940-50. Epub 2009/11/10.

[89] Lendahl U, Zimmerman LB, McKay RD. CNS stem cells express a new class of intermediate filament protein. Cell. 1990;60(4):585-95. Epub 1990/02/23.

[90] Suzuki S, Namiki J, Shibata S, Mastuzaki Y, Okano H. The neural stem/progenitor cell marker nestin is expressed in proliferative endothelial cells, but not in mature vasculature. The journal of histochemistry and cytochemistry : official journal of the Histochemistry Society. 2010;58(8):721-30. Epub 2010/04/28.

[91] Davidoff MS, Middendorff R, Enikolopov G, Riethmacher D, Holstein AF, Muller D. Progenitor cells of the testosterone-producing Leydig cells revealed. The Journal of cell biology. 2004;167(5):935-44. Epub 2004/12/01.

[92] Asahara T, Takahashi T, Masuda H, Kalka C, Chen D, Iwaguro H, et al. VEGF contributes to postnatal neovascularization by mobilizing bone marrow-derived endothelial progenitor cells. The EMBO journal. 1999;18(14):3964-72. Epub 1999/07/16.

[93] Shaked Y, Ciarrocchi A, Franco M, Lee CR, Man S, Cheung AM, et al. Therapy-induced acute recruitment of circulating endothelial progenitor cells to tumors. Science. 2006;313(5794):1785-7. Epub 2006/09/23.

[94] Wickersheim A, Kerber M, de Miguel LS, Plate KH, Machein MR. Endothelial progenitor cells do not contribute to tumor endothelium in primary and metastatic tu-

mors. International journal of cancer Journal international du cancer. 2009;125(8): 1771-7. Epub 2009/07/08.

[95] Peters BA, Diaz LA, Polyak K, Meszler L, Romans K, Guinan EC, et al. Contribution of bone marrow-derived endothelial cells to human tumor vasculature. Nature medicine. 2005;11(3):261-2. Epub 2005/02/22.

[96] Purhonen S, Palm J, Rossi D, Kaskenpaa N, Rajantie I, Yla-Herttuala S, et al. Bone marrow-derived circulating endothelial precursors do not contribute to vascular endothelium and are not needed for tumor growth. Proceedings of the National Academy of Sciences of the United States of America. 2008;105(18):6620-5. Epub 2008/04/30.

[97] Nolan DJ, Ciarrocchi A, Mellick AS, Jaggi JS, Bambino K, Gupta S, et al. Bone marrow-derived endothelial progenitor cells are a major determinant of nascent tumor neovascularization. Genes & development. 2007;21(12):1546-58. Epub 2007/06/19.

[98] Suzuki K, Sun R, Origuchi M, Kanehira M, Takahata T, Itoh J, et al. Mesenchymal stromal cells promote tumor growth through the enhancement of neovascularization. Mol Med. 2011;17(7-8):579-87. Epub 2011/03/23.

[99] Klopp AH, Zhang Y, Solley T, Amaya-Manzanares F, Marini F, Andreeff M, et al. Omental adipose tissue-derived stromal cells promote vascularization and growth of endometrial tumors. Clinical cancer research : an official journal of the American Association for Cancer Research. 2012;18(3):771-82. Epub 2011/12/15.

[100] Zhu W, Huang L, Li Y, Qian H, Shan X, Yan Y, et al. Mesenchymal stem cell-secreted soluble signaling molecules potentiate tumor growth. Cell Cycle. 2011;10(18): 3198-207. Epub 2011/09/09.

[101] Shinagawa K, Kitadai Y, Tanaka M, Sumida T, Onoyama M, Ohnishi M, et al. Stroma-directed imatinib therapy impairs the tumor-promoting effect of bone marrow-derived mesenchymal stem cells in an orthotopic transplantation model of colon cancer. International journal of cancer Journal international du cancer. 2013;132(4): 813-23. Epub 2012/07/24.

[102] Melero-Martin JM, Dudley AC. Concise review: Vascular stem cells and tumor angiogenesis. Stem Cells. 2011;29(2):163-8. Epub 2011/07/07.

[103] Watt SM, Gullo F, van der Garde M, Markeson D, Camicia R, Khoo CP, et al. The angiogenic properties of mesenchymal stem/stromal cells and their therapeutic potential. British medical bulletin. 2013;108:25-53. Epub 2013/10/25.

[104] Tang Z, Wang A, Yuan F, Yan Z, Liu B, Chu JS, et al. Differentiation of multipotent vascular stem cells contributes to vascular diseases. Nature communications. 2012;3:875. Epub 2012/06/08.

[105] Xin HB, Deng KY, Rishniw M, Ji G, Kotlikoff MI. Smooth muscle expression of Cre recombinase and eGFP in transgenic mice. Physiological genomics. 2002;10(3):211-5. Epub 2002/09/05.

[106] Belteki G, Haigh J, Kabacs N, Haigh K, Sison K, Costantini F, et al. Conditional and inducible transgene expression in mice through the combinatorial use of Cre-mediated recombination and tetracycline induction. Nucleic acids research. 2005;33(5):e51. Epub 2005/03/24.

[107] McDonald OG, Owens GK. Programming smooth muscle plasticity with chromatin dynamics. Circulation research. 2007;100(10):1428-41. Epub 2007/05/26.

[108] McDonald OG, Wamhoff BR, Hoofnagle MH, Owens GK. Control of SRF binding to CArG box chromatin regulates smooth muscle gene expression in vivo. The Journal of clinical investigation. 2006;116(1):36-48. Epub 2006/01/06.

[109] Cao D, Wang Z, Zhang CL, Oh J, Xing W, Li S, et al. Modulation of smooth muscle gene expression by association of histone acetyltransferases and deacetylases with myocardin. Molecular and cellular biology. 2005;25(1):364-76. Epub 2004/12/17.

[110] Sengupta N, Seto E. Regulation of histone deacetylase activities. Journal of cellular biochemistry. 2004;93(1):57-67. Epub 2004/09/08.

[111] Zhou B, Margariti A, Zeng L, Xu Q. Role of histone deacetylases in vascular cell homeostasis and arteriosclerosis. Cardiovascular research. 2011;90(3):413-20. Epub 2011/01/15.

[112] Liu J, Wang Y, Wu Y, Ni B, Liang Z. Sodium Butyrate Promotes the Differentiation of Rat Bone Marrow Mesenchymal Stem Cells to Smooth Muscle Cells through Histone Acetylation. PloS one. 2014;9(12):e116183. Epub 2014/12/31.

[113] Surcel C, Savu C, Chibelean C, Iordache A, Mirvald C, Sinescu I. Comparative analysis of different surgical procedures for female stress urinary incontinence. Is stem cell implantation the future? Romanian journal of morphology and embryology = Revue roumaine de morphologie et embryologie. 2012;53(1):151-4. Epub 2012/03/08.

[114] Wang KC, Helms JA, Chang HY. Regeneration, repair and remembering identity: the three Rs of Hox gene expression. Trends in cell biology. 2009;19(6):268-75. Epub 2009/05/12.

[115] Lumsden A, Krumlauf R. Patterning the vertebrate neuraxis. Science. 1996;274(5290): 1109-15. Epub 1996/11/15.

[116] Akam M. Hox genes: from master genes to micromanagers. Current biology : CB. 1998;8(19):R676-8. Epub 1998/10/13.

[117] Ackema KB, Charite J. Mesenchymal stem cells from different organs are characterized by distinct topographic Hox codes. Stem cells and development. 2008;17(5): 979-91. Epub 2008/06/07.

[118] Liedtke S, Buchheiser A, Bosch J, Bosse F, Kruse F, Zhao X, et al. The HOX Code as a "biological fingerprint" to distinguish functionally distinct stem cell populations derived from cord blood. Stem cell research. 2010;5(1):40-50. Epub 2010/05/04.

[119] Miano JM, Firulli AB, Olson EN, Hara P, Giachelli CM, Schwartz SM. Restricted expression of homeobox genes distinguishes fetal from adult human smooth muscle cells. Proceedings of the National Academy of Sciences of the United States of America. 1996;93(2):900-5. Epub 1996/01/23.

[120] Shen X, Yu L, Weir JW, Gorovsky MA. Linker histones are not essential and affect chromatin condensation in vivo. Cell. 1995;82(1):47-56. Epub 1995/07/14.

[121] Jedrusik MA, Schulze E. A single histone H1 isoform (H1.1) is essential for chromatin silencing and germline development in Caenorhabditis elegans. Development. 2001;128(7):1069-80. Epub 2001/03/14.

[122] Bustin M, Catez F, Lim JH. The dynamics of histone H1 function in chromatin. Molecular cell. 2005;17(5):617-20. Epub 2005/03/08.

[123] Bostrom K, Tintut Y, Kao SC, Stanford WP, Demer LL. HOXB7 overexpression promotes differentiation of C3H10T1/2 cells to smooth muscle cells. Journal of cellular biochemistry. 2000;78(2):210-21. Epub 2000/06/08.

[124] Potta SP, Liang H, Pfannkuche K, Winkler J, Chen S, Doss MX, et al. Functional characterization and transcriptome analysis of embryonic stem cell-derived contractile smooth muscle cells. Hypertension. 2009;53(2):196-204. Epub 2008/12/10.

[125] Pruett ND, Visconti RP, Jacobs DF, Scholz D, McQuinn T, Sundberg JP, et al. Evidence for Hox-specified positional identities in adult vasculature. BMC developmental biology. 2008;8:93. Epub 2008/10/02.

[126] Majesky MW. Developmental basis of vascular smooth muscle diversity. Arteriosclerosis, thrombosis, and vascular biology. 2007;27(6):1248-58. Epub 2007/03/24.

[127] Guo X, Stice SL, Boyd NL, Chen SY. A novel in vitro model system for smooth muscle differentiation from human embryonic stem cell-derived mesenchymal cells. American journal of physiology Cell physiology. 2013;304(4):C289-98. Epub 2012/12/12.

[128] Park WS, Heo SC, Jeon ES, Hong da H, Son YK, Ko JH, et al. Functional expression of smooth muscle-specific ion channels in TGF-beta(1)-treated human adipose-derived mesenchymal stem cells. American journal of physiology Cell physiology. 2013;305(4):C377-91. Epub 2013/06/14.

[129] To C, Farnsworth RH, Vail ME, Chheang C, Gargett CE, Murone C, et al. Hypoxia-controlled EphA3 marks a human endometrium-derived multipotent mesenchymal stromal cell that supports vascular growth. PloS one. 2014;9(11):e112106. Epub 2014/11/25.

[130] Espagnolle N, Guilloton F, Deschaseaux F, Gadelorge M, Sensebe L, Bourin P. CD146 expression on mesenchymal stem cells is associated with their vascular smooth muscle commitment. Journal of cellular and molecular medicine. 2014;18(1):104-14. Epub 2013/11/06.

[131] Passman JN, Dong XR, Wu SP, Maguire CT, Hogan KA, Bautch VL, et al. A sonic hedgehog signaling domain in the arterial adventitia supports resident Sca1+ smooth muscle progenitor cells. Proceedings of the National Academy of Sciences of the United States of America. 2008;105(27):9349-54. Epub 2008/07/02.

[132] Campagnolo P, Cesselli D, Al Haj Zen A, Beltrami AP, Krankel N, Katare R, et al. Human adult vena saphena contains perivascular progenitor cells endowed with clonogenic and proangiogenic potential. Circulation. 2010;121(15):1735-45. Epub 2010/04/07.

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