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Review

Vascular wall-resident stem cells

Diana Klein[1], Hans-Peter Hohn[1], Veronika Kleff[1], Derya Tilki[2,3] and Süleyman Ergün[1]

[1]Institute of Anatomy, University Hospital Essen, Essen, Germany, [2]Howard Hughes Medical Institute, Ansary Center for Stem Cell Therapeutics, and Department of Genetic Medicine, Weill Medical College of Cornell University, New York, USA, [3]Department of Urology, University Hospital Großhadern, Munich, Germany

Summary. New vessels in the adult have been considered to be formed not only by angiogenesis, but also by postnatal vasculogenesis via endothelial progenitor cells (EPCs). However, it is still a matter of debate as to what extent the EPCs contribute to new vessel formation in the adult. While the role of the circulating and bone marrow-derived EPCs has intensively been studied, the contribution of the vascular wall itself was neglected for a long time. Evidence published in the last few years strongly suggests the existence of different stem and progenitor cell types in the vascular wall. Particularly, the presence of EPCs and smooth muscle progenitor cells (SMPCs) in distinct zones of the vascular wall supports the hypothesis that not only BM- or C-EPCs, but also vascular wall-resident stem cells (VW-SCs) might contribute to new vessel formation and vascular wall morphogenesis. However, the differentiation potential of the VW-SCs, e.g. whether a VW-SC is able to give rise to a complete hierarchy of vascular progenitors still remains to be studied. This review will provide a survey about the VW-SCs and their potential impact in vascular biology.

Key words: Postnatal vascuologenesis, Angiogenesis, Tumor, Atherosclerosis, Vascular adventitia

Introduction

The *de novo* development of blood vessels from angioblasts/hemangioblats during embryogenesis and their subsequent stabilization by integration of peri-endothelial cells, as well as their hierarchic organization according to the demands on tissue blood supply are essential prerequisites for organogenesis in the fetal period. Similar processes also take place during tissue regeneration and repair in the adult (Conway et al., 2001; Carmeliet, 2004; Folkman, 2007). But the neovascularization is also crucially involved in development of diseases such as atherosclerosis or tumor growth and metastasis. Until more than a decade ago it was generally postulated that new vessel formation in the adult is only provided by sprouting of vessels from pre-existing blood vessels, called angiogenesis (Folkman et al., 1989; Risau, 1997). This concept has been crucially revised since the discovery of EPCs by Asahara et al. in 1997. Since this report, in a daily growing body of literature, bone marrow (BM-EPCs), peripheral and cord blood (C-EPCs) have been described to serve the sources for EPCs (Shi et al., 1998; Vasa et al., 2001; Asahara and Isner, 2002; Murayama and Asahara, 2002; Khurana and Simons, 2003). While the direct and indirect contribution of BM- and C-EPCs to new vessel formation in adults is still a matter of debate, emerging data published in the last few years suggest the existence of niches for vascular stem cells (VSCs) and, especially, EPCs outside the BM, such as in the wall of embryonic and fetal aorta and adult blood vessels, the so called vascular wall-resident EPCs (VW-EPCs), as well as in peripheral organs such as liver (Alessandri et al., 2001; Ingram et al., 2004, 2005; Tavian et al., 2005; Zengin et al., 2006; Aicher et al., 2007; Grenier et al., 2007; Invernici et al., 2007). Moreover, the vascular wall seems to harbour other types of progenitor cells and/or stem cells with a capability of differentiating to smooth muscle cells (SMC), fibroblasts and macrophages (Tintut et al., 2003; Tavian et al., 2005; Zengin et al., 2006; Passman et al., 2008). VSCs have been considered to reside in the sub-endothelial zone and within the vascular adventitia. Additionally, the vascular adventitia produces factors such as SDF-1, which acts as a chemo attractant for recruited BM-derived circulating cells and

Offprint requests to: Süleyman Ergün, Prof. Dr. med., Institute of Anatomy, University Hospital Essen, Hufelandstr. 55, D-45147 Essen, Germany. e-mail: sueleyman.erguen@uk-essen.de

guides them to the vascular adventitia. These cells, in turn, have been suggested to enhance angiogenic activities of mature endothelial cells (EC) via secretion of pro-angiogenic factors (Grunewald et al., 2006). It becomes clear that a glue composed of EPC and other types of progenitors and stem cells provides pro-angiogenic and pro-vasculogenic potential within the vascular wall, which is of relevance not only for repair and self-renewal of vascular cells or vasa vasorum, but also for local capacity of neovascularization in processes of diseases such as tumor growth and metastasis, growth of atherosclerotic plaques and revascularization of ischemic tissue. The understanding of this potential in the vascular wall is also relevant for therapeutic manipulations. Particularly, data published in the literature during the last 5-6 years strongly suggest that the distinct zones of the vascular wall harbour different types of stem and progenitor cells (Tintut et al., 2003; Tavian et al., 2005; Zengin et al., 2006; Ergun et al., 2007; Passman et al., 2008). In the following we will focus on different subsets of vascular wall-resident stem cells, such as vascular wall-resident EPCs (VW-EPCs), vascular wall-resident smooth muscle progenitor cells (VW-SMPCs) and vascular wall-resident mesenchymal stem cells (VW-MSCs).

EPC history and the hemangioblast concept

The first discovery of EPCs circulating in peripheral blood by Asahara et al. (1997) showed that these cells are recruited to the sites of active angiogenesis and crucially changed our notion about new vessel formation in the adult. Generally, today, there is no doubt about the existence of EPCs in the adult, but confusion still exists about to what extent they contribute to adult neovascularization. Meanwhile, recent data suggest that only a few EPCs are apparently sufficient to induce angiogenesis (Rafii and Lyden, 2008). EPCs are mostly defined as peripheral blood- or BM-derived mononuclear cells that show clonal expression, stemness characteristics, adherence to matrix molecules and are capable of differentiation into EC (Barber and Iruela-Arispe, 2006). One of the essential methods used for the identification of EPCs is flow cytometry. The most frequently studied markers are CD34, AC133, KDR (VEGFR-2), Tie-2, AcLDL and UEA-1 lectin (Asahara and Kawamoto, 2004; Barber and Iruela-Arispe, 2006), but none of them can be used alone because the majority of the used markers are also expressed in hematopoietic progenitor or hematopoietic stem cells (HSCs). This in fact makes it difficult to discriminate EPCs from HSCs. Thus, expression analyses combining a set of such markers are necessary. Despite such hurdles, the field of adult EPCs, and in this context also the regulation of postnatal vasculogenesis, have received huge attention in the last decade because of the expected use of EPCs, or cellular therapeutics containing EPCs, in the establishment of new clinical treatment procedures in vascular and ischemic disorders. Not only for the formation of new vessels, but also for the regeneration of other tissues and in the process of tissue engineering, EPCs seem to play a crucial role (Asahara and Kawamoto, 2004; Barber and Iruela-Arispe, 2006). Signalling mechanisms induced by EPCs/EC exert effects on neighbouring cells and tissues which have been reported to be indispensable for organ-specific internal architecture (Tepper et al., 2002; Asahara and Kawamoto, 2004).

According to the main body of the published literature, EPCs are thought to originate from bone marrow and circulate in peripheral blood (Asahara et al., 1997; Shi et al., 1998; Gehling et al., 2000; Ingram et al., 2005; Kovacic et al., 2008). During embryogenesis, blood vessels are formed *de novo* by the patterned assembly of angioblasts, the progenitor cells with capacity to differentiate to endothelial cells. This process, called vasculogenesis, is thought to be the basic mechanism leading to the formation of the aorta and its main branches. From the published findings it appears that newly-formed mesodermal cells migrate toward the yolk sac, where they differentiate to hematopoietic and endothelial precursor cells, the so called hemangioblasts/angioblasts which form the blood islands, an aggregation of mesodermal progenitors in the wall of the yolk sac (Risau and Flamme, 1995; Sabin, 2002). The cells of the outer layer of the blood islands change to a spindle morphology and then differentiate into ECs, while the majority of the inner cell mass differentiates into hematopoietic cells. The concept of hemangioblast as the common ancestral stem cell for both endothelial and hematopoietic cells has been discussed controversially over decades, but recently published findings strongly suggest the existence of such multipotent stem cells leading to the generation of endothelial and blood cells (Huber et al., 2004; Eilken et al., 2009). However, the adult hemangioblast has not been identified so far, while theoretically the existence of such an adult stem cell can be postulated, considering the fact that adult EPCs and HSCs or hematopoietic progenitors (HPCs) share common niches. Recently, published findings suggest that BM seems not to be the only niche where these stem or progenitor cells reside side by side. One of the emerging peripheral niches is apparently the vascular wall, which in the following will be presented in detail.

Vascular wall-resident vascular stem cells (VW-VSCs)

Superficially considered, the vascular wall is simply constructed by three layers, the so called tunica intima, media and adventitia (Fig. 1). The intima is constructed by endothelial cells, which are organized along the longitudinal axis of the vessels, and the sub-endothelial space containing few single cells, different types of collagen and extra cellular matrix. The tunica media is built up by smooth muscle cells which are organized concentrically and perpendicular to the endothelial cell

layer. Finally, the adventitia is composed mainly by fibroblasts and collagen material and, importantly, contains the so called vasa vasorum and nervous bundle modulating contractility of the vascular wall. For several decades, it was generally accepted that adult vascular wall cells were differentiated and postmitotic and thus, mainly quiescent cells. This view has changed by the findings obtained from angiogenesis research which clearly revealed that mature endothelial cells can be activated to proliferate and form new vessels (Risau, 1997; Carmeliet and Collen, 2000; Folkman, 2003). For a long time, this process was thought to be the only mechanism leading to new vessel formation in the adult because of assumed absence of vascular stem cells in the adult. But already in 1988 in the *Textbook of Histology* Simionescu and Simionescu mentioned that "in the partition between internal media and surrounding tissues and organs, the connective tissue cells of the vascular wall" contain a pluripotential pool for cell formation and local repair, for example, in endothelial regeneration (Simionescu and Simionescu, 1988). Recent findings obtained from *in vitro* studies on human material and *in vivo* studies on animal models using different techniques confirm the existence of potentially pluri-/multipotent progenitor and stem cells in the vascular wall, particularly in the vascular adventitia (Fig. 1). While it has still not been explored whether a putative stem cell with the capacity to differentiate to all types of mature vascular wall cells does exist within the vessel wall, emerging findings presented in the last few years suggest the presence of different types of progenitor and stem cells within the vessel wall, which have the capacity to differentiate to mature vascular wall cells and thus will be called vascular wall-resident vascular stem cells (VW-VSCs). VW-VSCs encompass subtypes of progenitors such as VW-EPCs, VW-SMPCs and VW-MSCs.

Vascular wall-resident endothelial progenitor cells (VW-EPCs)

Besides the already described niches for EPCs such as BM and peripheral blood, in the last few years it has become clearer that the vascular wall harbours EPCs, as was initially shown for human embryonic aorta (Alessandri et al., 2001). Further studies revealed the presence of a complete hierarchy of EPCs in the wall of human adult blood vessels and umbilical cord blood (Ingram et al., 2004, 2005). The exact location of the EPCs within the vascular wall was identified in distinct zones of the vessel wall, as shown in the sub-endothelial space and the so called "vasculogenic zone" (Fig. 1) within the vascular adventitia in close vicinity to the tunica media (Zengin et al., 2006). So far it has not been conclusively shown whether VW-EPCs can be mobilized from the adventitia or the sub-endothelial space into peripheral blood. But it was recently demonstrated that EPCs derived from peripheral organs such as liver or intestine contribute to the pool of circulating EPCs (Aicher et al., 2007). Since it was reported that intra organ large arteries and veins of several organs, among them also blood vessels of the liver, clearly contain VW-EPCs within their adventitia (Zengin et al., 2006) it can be speculated that at least a part of EPCs coming from

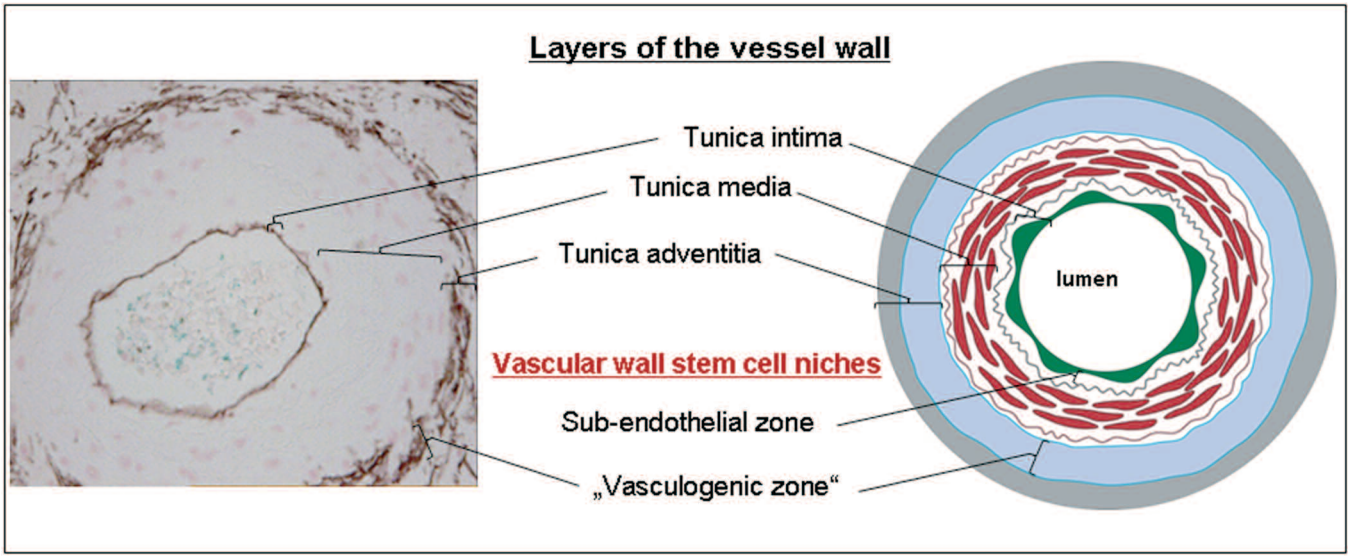

Fig. 1. Immunostaining for CD34 on a section of pulmonal artery shows exemplarily positive staining in the mature endothelium as expected, but also a localization of CD34(+) positive cells in the adventitia of this normal artery. The graphic presented in the right panel shows the layers of the vessel wall and the so far indentified stem cell niches of the vascular wall, such as the so-called vasculogenic zone in the adventitia and the the sub-endothelial space.

these organs might be mobilized from the wall of their blood vessels. There, EPCs may enter the circulation either through the vascular wall or via vasa vasorum. Supporting the VW-EPC hypothesis, more recently it was shown that the EPCs identified in the adipose tissue were localized in the adipose tissue blood vessels when the adipose tissue was separated in blood vessel fraction and adipose tissue without blood vessels (Grenier et al., 2007). Furthermore, the authors showed that EPCs mobilized from micro vessels of adipose tissue contribute to the vascularization and tissue regeneration (Grenier et al., 2007). Data presented by different groups demonstrate conformingly that vessel walls contain CD34(+)VEGFR-2(+)CD31(-)CD144(-) cells, and interestingly, these cells become positive for CD144 once they are activated to outgrow from the vessel wall (Alessandri et al., 2001; Zengin et al., 2006; Pasquinelli et al., 2007). Taken together, BM, peripheral and cord blood, some organs like liver and intestine, as well as the wall of middle sized/large blood vessels, serve as sources for EPCs. Particularly, the identification of the vessel wall as a niche for EPCs and other progenitors is of essential impact for vascular biology and has functional and therapeutic consequences regarding our notion about tissue vascularization in health and diseases. From different body regions fragments of veins and arteries can surgically be extracted without unacceptable burden for patients, which subsequently can be used to isolate EPCs and expand them *in vitro* under specific culture conditions. These cells then can be re-implanted to the same patient in a further step and might help to establish patient specific cellular therapeutics in the treatment of vascular and ischemic disorders. Of course, today this conception is a hypothesis and we are still far from the exact identification and cellular characterization of the VW-EPCs, but this vision is more than utopia. Indirect hints for involvement of the VW-EPCs in vascular repair have been obtained from *in vivo* studies. Using eNOS-deficient mice in which BM was replaced by BM cells from EGFP(+) wild type mice, no integration of EGFP(+) EPCs into the vascular endothelial layer was observed, while a small part of EGFP(+) BM cells were found in the vascular adventitia (Perry et al., 2009). From these results the authors conclude that renewal of chronic dysfunctional endothelium and endothelial homeostasis may be dependent on resident vascular progenitor cells. Supporting this interpretation, it was shown that in the initial phase of tumor vascularization a considerable part of tumor vessels was formed by EPCs of BM origin, while in later stages of tumor development BM originated vessels were diluted by vessels formed probably by endothelial cells coming from peripheral niches (Nolan et al., 2007), e.g. from the vessel wall itself.

Symbiotic co-existance of EPCs and HPCs in the vascular wall

The symbiotic fate of hematopoiesis and vasculogenesis is underlined by the hemangioblasts, which have been considered to be the ancestral embryonic precursor cells for endothelial and hematopoietic cells (Risau et al., 1988; Bollerot et al., 2005; Tavian et al., 2005). Hemopoietic endothelial cells positive for transcription factor Runx1 have been shown to represent a subpopulation of the yolk sac endothelium. The corresponding region within the dorsal aorta is called hemogenic endothelium and generates definitive hematopoietic cells including hematopoietic stem cells (Ribatti, 2008). It has previously been shown that embryonic cells expressing endothelial molecules can generate blood cells. Recently, Schroeder and co-workers showed that haemogenic endothelial cells give rise to blood cells using continuous long-term single-cell observation of mouse mesodermal cells (Eilken et al., 2009).

Primitive hematopoietic cells share with EPCs the expression of a great part of the cell surface markers, such as c-kit, CD133, Sca-1, VE-cadherin, VEGFR-2 and endoglin (Rafii and Lyden, 2003; Kopp et al., 2006; Case et al., 2007). Functionally, the concerted action of both HPCs and EPCs is essentially involved in the regulation of tumor vascularization. VEGF, secreted by tumor cells, recruits VEGFR-1(+) HPCs and VEGFR-2(+) EPCs to the tumor tissue and activates tumor neoangiogenesis (Lyden et al., 2001). How necessary this interaction between HPCs and EPCs is for tumor growth was shown in an Id-mutant mouse model, where tumor progression did not take place because of absent mobilization of BM-derived progenitors and defective angiogenesis. Tumor growth and angiogenesis were restored by transplantation of wild-type BM or VEGF-mobilized stem cells (Lyden et al., 1999). HPCs recruited to the tumor tissue in turn release pro-angiogenic factors, such as VEGF-A, angiopoietins and PDGF, which recruit the EPCs to the tumor vessels (Okamoto et al., 2005; Grunewald et al., 2006). Moreover, via these mechanisms HPCs may also promote the assembly of peri-endothelial cells into the vascular wall, stabilizing new vessels. Emerging data suggest that besides the BM, HPCs might also reside within the vascular wall. Using flow cytometric and immunohistochemical analyses CD45(+)CD34(-) cells have been shown to be present in the adult arterial wall (Fig. 2), suggesting the potential existence of vascular wall-resident HPCs (VW-HPCs) (Zengin et al., 2006). The fact that these cells were found to be predominantly localized in a zone of the vascular adventitia where CD34(+)Tie-2(+)VEGFR-2(+)VE-cad(-) VW-EPCs reside (Zengin et al., 2006) again indicates the symbiotic co-existence of both VW-HPCs and VW-EPCs in the vascular wall, as in adult BM or in yolk sack during embryogenesis (Risau et al., 1988; Tavian et al., 2005). Peault and co-workers demonstrated that a population of VEGFR-2(+) angiohematopoietic mesodermal stem cells migrate from the paraaortic splanchnopleura into the ventral part of the aorta, where they give rise to endothelial and hematopoietic cells (Tavian et al., 2005). Supporting these findings it was shown that CD68(+)

macrophages can be accumulated in the vascular adventitia during *ex vivo* arterial ring assay and *in vivo* in a rat arteriogenesis model, despite experimental depletion of the BM (Zengin et al., 2006). Based on the aforementioned findings it is conceivable to hypothesize a) that the symbiotic co-existence of both HPCs and EPCs is not limited to the embryonic period and rather exists throughout the entire lifetime, and b) that outside the BM HPCs reside within the vascular wall side by side with EPCs. Recent findings interestingly show that vascular stem cells colonize the vascular adventitia during the embryonic and fetal period (Passman et al., 2008). One could postulate that for both HPCs and EPCs BM, peripheral blood and vascular wall serve as niches which might be interconnected to each other dynamically, contributing to a balanced movement of the progenitor cells between BM and vascular adventitia via blood circulation.

Vascular wall-resident smooth muscle cell progenitor cells (VW-SMPCs)

While the initial step of new vessel formation is provided by endothelial cells which build up a nascent network of immature vessels, their maintenance and further functional and morphological maturation essentially depend on the integration of pericytes or smooth muscle cells into the vessel wall which cover the endothelial cells. These contractile elements of the vessel wall are mainly generated from the mesenchymal cells during embryogenesis, but in some parts of the vessels they have been shown to be derived from the neural crest material (Jiang et al., 2000). Thus, within the same blood vessel the origin of smooth muscle cells can be heterogeneous. Vascular stabilization is also needed for newly formed adult vessels in order to survive and to function. Thus, it is of relevance to identify the origin of adult progenitor and stem cells giving rise to vascular smooth muscle cells. Data published in the literature so far suggest BM (Sata et al., 2002), peripheral blood (Simper et al., 2002), adipose tissue (Rodriguez et al., 2006), skeletal muscle (Majka et al., 2003), hair follicle (Liu et al., 2008) and finally the vessel wall as niches for smooth muscle progenitors (Hu et al., 2004; Sainz et al., 2006; Zengin et al., 2006; Passman et al., 2008). A part of the published findings identify BM-derived MSCs and an immature type of pericytes isolated from the aortic wall to give rise to smooth muscle cells (Sata et al., 2002; Howson et al., 2005), whereas further strong evidence suggests the generation of vascular smooth muscle cells from MSCs and/or VSCs residing in the vascular adventitia (Hu et al., 2004; Zengin et al., 2006; Pasquinelli et al., 2007; Passman et al., 2008). Using the human internal thoracic artery in sprouting assay it was shown that smooth muscle actin (α-SMA) positive cells were mobilized from the vessel wall and closely assembled to the endothelial sprouts (Zengin et al., 2006). Supporting these findings, using a genetic model for sonic hedgehog signalling domain Passman et al. recently demonstrated the existence of Sca1(+) vascular stem cells in the arterial adventitia which were capable of differentiating into SMC (Passman et al., 2008). Interestingly, the Sca1(+) vascular stem cells are exactly localized in the "vasculogenic zone", which has been shown to harbour EPCs and HPCs (Zengin et al., 2006). Together, these data strongly suggest that apparently the wall of large or small arteries and veins of different species, particularly the vascular adventitia, contains VW-SMPCs and MSCs with the capability to differentiate to mature vascular smooth muscle cells (Fig. 2). The functional relevance of the VW-SMPCs can be fixed on morphogenesis of new adult vessels and on their potential contribution to the formation of neointima in atherosclerotic process. VW-SMPCs might help to stabilize newly formed adult vessels by angiogenesis and postnatal vasculogenesis

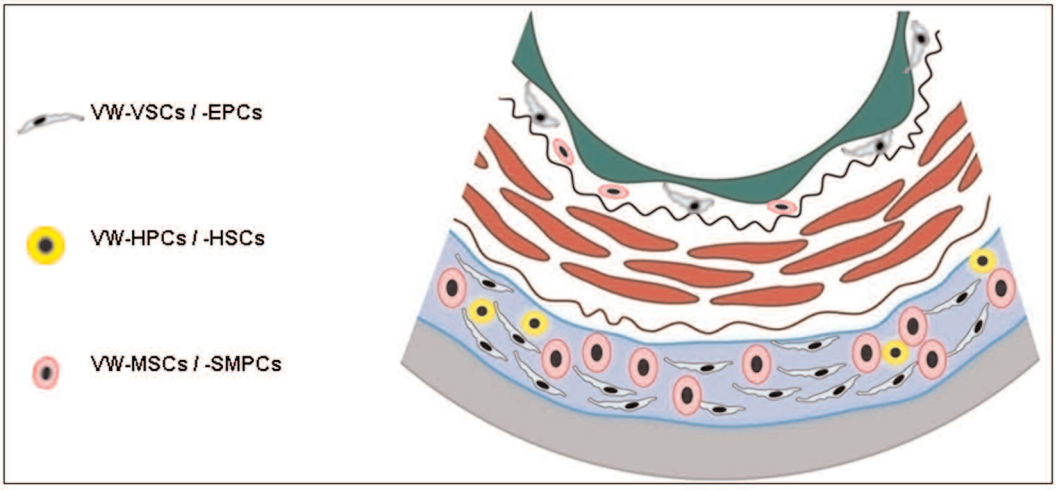

Fig. 2. Detailed graphical representation of the stem cell niches of the vascular wall mentioned in figure 1 with stem and progenitor cell types identified until now.

induced by ischemic disorders. Vascular stabilization is essential in order to ensure blood flow depending on tissue demand. But on the other hand, very recently published findings demonstrate that smooth muscle cells forming neointima after balloon-induced injury are apparently not generated from the BM-derived progenitors, but rather from the pre-existing vascular wall cells (Rodriguez-Menocal et al., 2009). These data again underline the clinical relevance of the local progenitor and stem cells residing in the vessel wall.

Vascular wall-resident MSCs (VW-MSCs)

Besides the EPCs and HSCs, adult arteries of animals and humans may contain cells with characteristics of ancestral stem cells. The blood vessel wall harbours a reservoir of progenitor cells that may be integral to the origin of the elusive MSCs and other related adult stem cells (Shi and Gronthos, 2003; Hu et al., 2004; Howson et al., 2005; Sainz et al., 2006; Covas et al., 2008; Crisan et al., 2008; Khan et al., 2008). Almost every organ seems to contain MSC, and increasing data suggest that MSC also reside within distinct zones of the vascular wall, such as sub-endothelial space and vascular adventitia (da Silva et al., 2006; Zengin et al., 2006; Ergun et al., 2008). In the following, these cells will be called VW-MSCs. The establishment of a VW-MSC niche in the vascular adventitia provides the basis for the rational design of additional in vivo therapeutic approaches. Moreover, there is evidence suggesting a perivascular location for VW-MSCs, correlating these cells with pericytes (Caplan, 1991, 2008). In this model the perivascular zone is the MSC niche *in vivo*, where local cues coordinate the transition to progenitor and mature cell phenotypes. However, their precise native localization remains obscure. The majority of data related to the contribution of MSCs to the formation of blood vessels is obtained by studies on BM-derived MSCs. In BM, the MSCs belong to the non-hematopoietic cells and only represent a small percentage of the marrow cells (Caplan, 1991). Under specific culture conditions the BM-derived MSCs differentiate into fat, muscle, bone and cartilage cells (Pittenger et al., 1999; Hirschi and Goodell, 2002; Song and Tuan, 2004). BM-derived MSCs also differentiate into vascular smooth muscle cells (SMC) (Kashiwakura et al., 2003; Zuk et al., 2001). After being seeded on a synthetic vascular graft, they differentiate into both SMC and EC in vivo (Matsumura et al., 2003).

EC and pericytes, as well as smooth muscle cells, cause a special microenvironment that affects the behaviour of stem cells, in a concept termed "vascular niche", and the basement membrane plays a central role in that niche (Palmer et al., 2000; Carmeliet, 2003; Nikolova et al., 2007). About 15 years ago the site of pericytes associated with vessels was suggested to be a peri-vascular niche for MSC (Nehls et al., 1992; Nehls and Drenckhahn, 1993). In the vascular wall, pericyte-like cells were shown to serve as cellular potential for VW-MSCs, which apparently differentiate into cell colonies expressing adipogenic, osteogenic and chondrogenic markers (Romanov et al., 2003) as BM-derived MSCs. According to this hypothesis, these pericyte-like cells are present in the subendothelial space of all vessels (Andreeva et al., 1998). Furthermore, recently it was shown that α-SMA expressing cells (Zengin et al., 2006) are closely associated to sprouting EC, and it was postulated that these α-SMA positive cells were probably generated from VW-MSCs. Furthermore, these cells were found to cover newly formed endothelial sprouts. Taken together VW-MSC might build up an adult multipotent stem cell population distributed through the whole body because of their close association to the blood vessels. With this property they might serve a cellular potential giving rise to not only endothelial and smooth muscle cells, but also to several other mature non-vascular cell types.

Conclusions

As recapitulated above, the data published in the literature, particularly those in the last few years strongly suggest the vascular wall as a niche for different types of stem and progenitor cell types. A rapidly emerging concept is that the wall of blood vessels itself provides a micro milieu serving for retrieval, integration, storage, and release of key regulators, including stem and/or progenitor cells. Based on the findings summarized in this review one can hypothesize that a cell type, normally involved in physiological vascular homeostasis, might also act as a reservoir of undifferentiated cells ready to supply the cellular demands of the tissue they belong to and acquiring local phenotypic characteristics. In response to stress, development of atherosclerotic plaques or injury, resident adventitial cells can be activated and specified to exhibit different functional and structural behaviours. Although it is still not clear whether the same maternal or progenitor cells give rise to the generation of mature pericytes, endothelial and smooth muscle cells, macrophages and blood cells from the vascular wall, the existence of such stem and progenitors in distinct zones of the vascular wall encourages the undertaking further effort in order to better characterize these cells and to evaluate their therapeutic potential.

References

Aicher A., Rentsch M., Sasaki K., Ellwart J.W., Fandrich F., Siebert R., Cooke J.P., Dimmeler S. and Heeschen C. (2007). Nonbone marrow-derived circulating progenitor cells contribute to postnatal neovascularization following tissue ischemia. Circ. Res. 100, 581-589.

Alessandri G., Girelli M., Taccagni G., Colombo A., Nicosia R., Caruso A., Baronio M., Pagano S., Cova L. and Parati E. (2001). Human vasculogenesis ex vivo: embryonal aorta as a tool for isolation of endothelial cell progenitors. Lab. Invest. 81, 875-885.

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

Asahara T. and Isner J.M. (2002). Endothelial progenitor cells for vascular regeneration. J. Hematother. Stem Cell Res. 11, 171-178.

Asahara T. and Kawamoto A. (2004). Endothelial progenitor cells for postnatal vasculogenesis. Am. J. Physiol Cell Physiol 287, C572-C579.

Asahara T., Murohara T., Sullivan A., Silver M., van der Z.R., Li T., Witzenbichler B., Schatteman G. and Isner J.M. (1997). Isolation of putative progenitor endothelial cells for angiogenesis. Science 275, 964-967.

Barber C.L. and Iruela-Arispe M.L. (2006). The ever-elusive endothelial progenitor cell: identities, functions and clinical implications. Pediatr. Res. 59, 26R-32R.

Bollerot K., Pouget C. and Jaffredo T. (2005). The embryonic origins of hematopoietic stem cells: a tale of hemangioblast and hemogenic endothelium. APMIS 113, 790-803.

Caplan A.I. (1991). Mesenchymal stem cells. J. Orthop. Res. 9, 641-650.

Caplan A.I. (2008). All MSCs are pericytes? Cell Stem Cell 3, 229-230.

Carmeliet P. (2003). Angiogenesis in health and disease. Nat. Med. 9, 653-660.

Carmeliet P. (2004). Manipulating angiogenesis in medicine. J. Intern. Med. 255, 538-561.

Carmeliet P. and Collen D. (2000). Molecular basis of angiogenesis. Role of VEGF and VE-cadherin. Ann. N. Y. Acad. Sci. 902, 249-262.

Case J., Mead L.E., Bessler W.K., Prater D., White H.A., Saadatzadeh M.R., Bhavsar J.R., Yoder M.C., Haneline L.S. and Ingram D.A. (2007). Human CD34+AC133+VEGFR-2+ cells are not endothelial progenitor cells but distinct, primitive hematopoietic progenitors. Exp. Hematol. 35, 1109-1118.

Conway E.M., Collen D. and Carmeliet P. (2001). Molecular mechanisms of blood vessel growth. Cardiovasc. Res. 49, 507-521.

Covas D.T., Panepucci R.A., Fontes A.M., Silva W.A. Jr, Orellana M.D., Freitas M.C., Neder L., Santos A.R., Peres L.C., Jamur M.C. and Zago M.A. (2008). Multipotent mesenchymal stromal cells obtained from diverse human tissues share functional properties and gene-expression profile with CD146+ perivascular cells and fibroblasts. Exp. Hematol. 36, 642-654.

Crisan M., Yap S., Casteilla L., Chen C.W., Corselli M., Park T.S., Andriolo G., Sun,B., Zheng B., Zhang L., Norotte C., Teng P.N., Traas J., Schugar R., Deasy B.M., Badylak S., Buhring H.J., Giacobino J.P., Lazzari L., Huard J. and Peault B. (2008). A perivascular origin for mesenchymal stem cells in multiple human organs. Cell Stem Cell 3, 301-313.

da Silva M.L., Chagastelles P.C. and Nardi N.B. (2006). Mesenchymal stem cells reside in virtually all post-natal organs and tissues. J. Cell Sci. 119, 2204-2213.

Eilken H.M., Nishikawa S. and Schroeder T. (2009). Continuous single-cell imaging of blood generation from haemogenic endothelium. Nature 457, 896-900.

Ergun S., Hohn H.P., Kilic N., Singer B.B. and Tilki D. (2008). Endothelial and hematopoietic progenitor cells (EPCs and HPCs): hand in hand fate determining partners for cancer cells. Stem Cell Rev. 4, 169-177.

Ergun S., Tilki D., Hohn H.P., Gehling U. and Kilic N. (2007). Potential implications of vascular wall resident endothelial progenitor cells. Thromb. Haemost. 98, 930-939.

Folkman J. (2003). Angiogenesis and apoptosis. Semin. Cancer Biol. 13, 159-167.

Folkman J. (2007). Angiogenesis: an organizing principle for drug discovery? Nat. Rev. Drug Discov. 6, 273-286.

Folkman J., Watson K., Ingber D. and Hanahan D. (1989). Induction of angiogenesis during the transition from hyperplasia to neoplasia. Nature 339, 58-61.

Gehling U.M., Ergun S., Schumacher U., Wagener C., Pantel K., Otte M., Schuch G., Schafhausen P., Mende T., Kilic N., Kluge K., Schafer B., Hossfeld D.K. and Fiedler W. (2000). In vitro differentiation of endothelial cells from AC133-positive progenitor cells. Blood 95, 3106-3112.

Grenier G., Scime A., Le G.F., Asakura A., Perez-Iratxeta C., Andrade-Navarro M.A., Labosky P.A. and Rudnicki M.A. (2007). Resident endothelial precursors in muscle, adipose, and dermis contribute to postnatal vasculogenesis. Stem Cells 25, 3101-3110.

Grunewald M., Avraham I., Dor,Y., Bachar-Lustig E., Itin A., Jung S., Chimenti S., Landsman L., Abramovitch R. and Keshet E. (2006). VEGF-induced adult neovascularization: recruitment, retention, and role of accessory cells. Cell 124, 175-189.

Hirschi K.K. and Goodell M.A. (2002). Hematopoietic, vascular and cardiac fates of bone marrow-derived stem cells. Gene Ther. 9, 648-652.

Howson K.M., Aplin A.C., Gelati M., Alessandri G., Parati E.A. and Nicosia R.F. (2005). The postnatal rat aorta contains pericyte progenitor cells that form spheroidal colonies in suspension culture. Am. J. Physiol. Cell Physiol. 289, C1396-C1407.

Hu Y., Zhang Z., Torsney E., Afzal A.R., Davison F., Metzler B. and Xu Q. (2004). Abundant progenitor cells in the adventitia contribute to atherosclerosis of vein grafts in ApoE-deficient mice. J. Clin. Invest 113, 1258-1265.

Huber T.L., Kouskoff V., Fehling H.J., Palis J. and Keller G. (2004). Haemangioblast commitment is initiated in the primitive streak of the mouse embryo. Nature 432, 625-630.

Ingram D.A., Mead L.E., Tanaka H., Meade V., Fenoglio A., Mortell K., Pollok K., Ferkowicz M.J., Gilley D. and Yoder M.C. (2004). Identification of a novel hierarchy of endothelial progenitor cells using human peripheral and umbilical cord blood. Blood 104, 2752-2760.

Ingram D.A., Mead L.E., Moore D.B., Woodard W., Fenoglio A. and Yoder M.C. (2005). Vessel wall-derived endothelial cells rapidly proliferate because they contain a complete hierarchy of endothelial progenitor cells. Blood 105, 2783-2786.

Invernici G., Emanueli C., Madeddu P., Cristini S., Gadau S., Benetti A., Ciusani E., Stassi G., Siragusa M., Nicosia R., Peschle C., Fascio U., Colombo A., Rizzuti T., Parati E. and Alessandri G. (2007). Human fetal aorta contains vascular progenitor cells capable of inducing vasculogenesis, angiogenesis, and myogenesis in vitro and in a murine model of peripheral ischemia. Am. J. Pathol. 170, 1879-1892.

Jiang X., Rowitch D.H., Soriano P., McMahon A.P. and Sucov H.M. (2000). Fate of the mammalian cardiac neural crest. Development 127, 1607-1616.

Kashiwakura Y., Katoh Y., Tamayose K., Konishi H., Takaya N., Yuhara S., Yamada M., Sugimoto K. and Daida H. (2003). Isolation of bone marrow stromal cell-derived smooth muscle cells by a human SM22alpha promoter: in vitro differentiation of putative smooth muscle progenitor cells of bone marrow. Circulation 107, 2078-2081.

Khan W.S., Tew S.R., Adesida A.B. and Hardingham T.E. (2008).

Human infrapatellar fat pad-derived stem cells express the pericyte marker 3G5 and show enhanced chondrogenesis after expansion in fibroblast growth factor-2. Arthritis Res. Ther. 10, R74.

Khurana R. and Simons M. (2003). Endothelial progenitor cells: precursors for angiogenesis. Semin. Thorac. Cardiovasc. Surg. 15, 250-258.

Kopp H.G., Ramos C.A. and Rafii S. (2006). Contribution of endothelial progenitors and proangiogenic hematopoietic cells to vascularization of tumor and ischemic tissue. Curr. Opin. Hematol. 13, 175-181.

Kovacic J.C., Moore J., Herbert A., Ma D., Boehm M. and Graham R.M. (2008). Endothelial progenitor cells, angioblasts, and angiogenesis--old terms reconsidered from a current perspective. Trends Cardiovasc. Med. 18, 45-51.

Liu J.Y., Peng H.F. and Andreadis S.T. (2008). Contractile smooth muscle cells derived from hair-follicle stem cells. Cardiovasc. Res. 79, 24-33.

Lyden D., Hattori K., Dias S., Costa C., Blaikie P., Butros L., Chadburn A., Heissig B., Marks W., Witte L., Wu Y., Hicklin D., Zhu Z., Hackett N.R., Crystal R.G., Moore M.A., Hajjar K.A., Manova K., Benezra R. and Rafii S. (2001). Impaired recruitment of bone-marrow-derived endothelial and hematopoietic precursor cells blocks tumor angiogenesis and growth. Nat. Med. 7, 1194-1201.

Lyden D., Young A.Z., Zagzag D., Yan W., Gerald W., O'Reilly R., Bader B.L., Hynes R.O., Zhuang Y., Manova K. and Benezra R. (1999). Id1 and Id3 are required for neurogenesis, angiogenesis and vascularization of tumour xenografts. Nature 401, 670-677.

Majka S.M., Jackson K.A., Kienstra K.A., Majesky M.W., Goodell M.A. and Hirschi K.K. (2003). Distinct progenitor populations in skeletal muscle are bone marrow derived and exhibit different cell fates during vascular regeneration. J. Clin. Invest 111, 71-79.

Matsumura G., Miyagawa-Tomita S., Shin'oka T., Ikada Y. and Kurosawa H. (2003). First evidence that bone marrow cells contribute to the construction of tissue-engineered vascular autografts in vivo. Circulation 108, 1729-1734.

Murayama T. and Asahara T. (2002). Bone marrow-derived endothelial progenitor cells for vascular regeneration. Curr. Opin. Mol. Ther. 4, 395-402.

Nehls V. and Drenckhahn D. (1993). The versatility of microvascular pericytes: from mesenchyme to smooth muscle? Histochemistry 99, 1-12.

Nehls V., Denzer K. and Drenckhahn D. (1992). Pericyte involvement in capillary sprouting during angiogenesis in situ. Cell Tissue Res. 270, 469-474.

Nikolova G., Strilic B. and Lammert E. (2007). The vascular niche and its basement membrane. Trends Cell Biol. 17, 19-25.

Nolan D.J., Ciarrocchi A., Mellick A.S., Jaggi J.S., Bambino K., Gupta S., Heikamp E., McDevitt M.R., Scheinberg D.A., Benezra R. and Mittal V. (2007). Bone marrow-derived endothelial progenitor cells are a major determinant of nascent tumor neovascularization. Genes Dev. 21, 1546-1558.

Okamoto R., Ueno M., Yamada Y., Takahashi N., Sano H., Suda T. and Takakura N. (2005). Hematopoietic cells regulate the angiogenic switch during tumorigenesis. Blood 105, 2757-2763.

Palmer T.D., Willhoite A.R. and Gage F.H. (2000). Vascular niche for adult hippocampal neurogenesis. J. Comp. Neurol. 425, 479-494.

Pasquinelli G., Tazzari P.L., Vaselli C., Foroni L., Buzzi M., Storci G., Alviano F., Ricci F., Bonafe M., Orrico C., Bagnara G.P., Stella A. and Conte R. (2007). Thoracic aortas from multiorgan donors are suitable for obtaining resident angiogenic mesenchymal stromal cells. Stem Cells 25, 1627-1634.

Passman J.N., Dong X.R., Wu S.P., Maguire C.T., Hogan K.A., Bautch V.L. and Majesky M.W. (2008). A sonic hedgehog signaling domain in the arterial adventitia supports resident Sca1+ smooth muscle progenitor cells. Proc. Natl. Acad. Sci. USA 105, 9349-9354.

Perry T.E., Song M., Despres D.J., Kim S.M., San H., Yu Z.X., Raghavachari,N., Schnermann,J., Cannon R.O. III and Orlic D. (2009). Bone marrow-derived cells do not repair endothelium in a mouse model of chronic endothelial cell dysfunction. Cardiovasc. Res. 84, 317-325.

Pittenger M.F., Mackay A.M., Beck S.C., Jaiswal R.K., Douglas R., Mosca J.D., Moorman M.A., Simonetti D.W., Craig S. and Marshak D.R. (1999). Multilineage potential of adult human mesenchymal stem cells. Science 284, 143-147.

Rafii S. and Lyden D. (2003). Therapeutic stem and progenitor cell transplantation for organ vascularization and regeneration. Nat. Med. 9, 702-712.

Rafii S. and Lyden D. (2008). Cancer. A few to flip the angiogenic switch. Science 319, 163-164.

Ribatti D. (2008). Hemangioblast does exist. Leuk. Res. 32, 850-854.

Risau W. (1997). Mechanisms of angiogenesis. Nature 386, 671-674.

Risau W. and Flamme I. (1995). Vasculogenesis. Annu. Rev. Cell Dev. Biol. 11, 73-91.

Risau W., Sariola H., Zerwes H.G., Sasse J., Ekblom P., Kemler R. and Doetschman T. (1988). Vasculogenesis and angiogenesis in embryonic-stem-cell-derived embryoid bodies. Development 102, 471-478.

Rodriguez L.V., Alfonso Z., Zhang R., Leung J., Wu B. and Ignarro L.J. (2006). Clonogenic multipotent stem cells in human adipose tissue differentiate into functional smooth muscle cells. Proc. Natl. Acad. Sci. USA 103, 12167-12172.

Rodriguez-Menocal L., St-Pierre M., Wei Y., Khan S., Mateu D., Calfa M., Rahnemai-Azar A.A., Striker G., Pham S.M. and Vazquez-Padron R.I. (2009). The origin of post-injury neointimal cells in the rat balloon injury model. Cardiovasc. Res. 81, 46-53.

Romanov Y.A., Svintsitskaya V.A. and Smirnov V.N. (2003). Searching for alternative sources of postnatal human mesenchymal stem cells: candidate MSC-like cells from umbilical cord. Stem Cells 21, 105-110.

Sabin F.R. (2002). Preliminary note on the differentiation of angioblasts and the method by which they produce blood-vessels, blood-plasma and red blood-cells as seen in the living chick. 1917. J. Hematother. Stem Cell Res. 11, 5-7.

Sainz J., Al Haj Z.A., Caligiuri G., Demerens C., Urbain D., Lemitre M. and Lafont A. (2006). Isolation of "side population" progenitor cells from healthy arteries of adult mice. Arterioscler. Thromb. Vasc. Biol. 26, 281-286.

Sata M., Saiura A., Kunisato A., Tojo A., Okada S., Tokuhisa T., Hirai H., Makuuchi M., Hirata Y. and Nagai R. (2002). Hematopoietic stem cells differentiate into vascular cells that participate in the pathogenesis of atherosclerosis. Nat. Med. 8, 403-409.

Shi Q., Rafii S., Wu M.H., Wijelath E.S., Yu C., Ishida A., Fujita Y., Kothari S., Mohle R., Sauvage L.R., Moore M.A., Storb R.F. and Hammond W.P. (1998). Evidence for circulating bone marrow-derived endothelial cells. Blood 92, 362-367.

Shi S. and Gronthos S. (2003). Perivascular niche of postnatal mesenchymal stem cells in human bone marrow and dental pulp. J. Bone Miner. Res. 18, 696-704.

Simionescu N. and Simionescu M. (1988). Cell and tissue biology.

Urban & Schwarzenberg. Bolitmore Berlin.

Simper D., Stalboerger P.G., Panetta C.J., Wang S. and Caplice N.M. (2002). Smooth muscle progenitor cells in human blood. Circulation 106, 1199-1204.

Song L. and Tuan R.S. (2004). Transdifferentiation potential of human mesenchymal stem cells derived from bone marrow. FASEB J. 18, 980-982.

Tavian M., Zheng B., Oberlin E., Crisan M., Sun B., Huard J. and Peault B. (2005). The vascular wall as a source of stem cells. Ann. N. Y. Acad. Sci. 1044, 41-50.

Tepper O.M., Galiano R.D., Capla J.M., Kalka C., Gagne P.J., Jacobowitz G.R., Levine J.P. and Gurtner G.C. (2002). Human endothelial progenitor cells from type II diabetics exhibit impaired proliferation, adhesion, and incorporation into vascular structures. Circulation 106, 2781-2786.

Tintut Y., Alfonso Z., Saini T., Radcliff K., Watson K., Bostrom K. and Demer L.L. (2003b). Multilineage potential of cells from the artery wall. Circulation 108, 2505-2510.

Vasa M., Fichtlscherer S., Adler K., Aicher A., Martin H., Zeiher A.M. and Dimmeler S. (2001). Increase in circulating endothelial progenitor cells by statin therapy in patients with stable coronary artery disease. Circulation 103, 2885-2890.

Zengin E., Chalajour F., Gehling U.M., Ito W.D., Treede H., Lauke H., Weil J., Reichenspurner H., Kilic N. and Ergun S. (2006). Vascular wall resident progenitor cells: a source for postnatal vasculogenesis. Development 133, 1543-1551.

Zuk P.A., Zhu M., Mizuno H., Huang J., Futrell J.W., Katz A.J., Benhaim P., Lorenz H.P. and Hedrick M.H. (2001). Multilineage cells from human adipose tissue: implications for cell-based therapies. Tissue Eng. 7, 211-228.

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