Brief Review

Smooth Muscle Cell Origin and Its Relation to Heterogeneity in Development and Disease

Adriana C. Gittenberger-de Groot, Marco C. DeRuiter, Maarten Bergwerff, Robert E. Poelmann

Smooth muscle cells (SMC) of the vascular system form an intriguing population of cells that are relevant for maintaining vascular tone and function. They also play a key role in pathological processes in the vessel wall.

If we focus on the development of intimal thickening in the latter function, it is clear that even this pathological subset presents itself in various forms. That is, arteriosclerosis after hypertension, arteriosclerosis, and restenosis after percutaneous transluminal coronary angioplasty or coronary artery bypass grafting surgery have features in common as well as characteristics selective for each disease.

Relevant to an understanding of the above processes is the basic question of whether we are dealing either with a SMC heterogeneity in origin or with a spatiotemporal heterogeneity in expression of differentiation markers. To add to this complexity there is an increasing evidence that already committed and differentiated cells can transdifferentiate into another cell type. In studying SMC heterogeneity, a combination of these factors is likely.

It has been shown by several research groups that SMC heterogeneity exists within the vessel wall, varying from the adult rat to the human fetal population. These data are mainly based on in vitro cell culture studies.

A different approach is to study the intact vascular wall and expression of differentiation markers. This approach shows a change in gene expression patterns with normal maturation and with development of intimal thickening of the vessel wall. During development of intimal thickening in various settings, including physiological circumstances, thickening experimentally induced by a perivascular cuff, and atherosclerosis in humans, reexpression of fetal genes has been observed as well as altered migration and proliferation patterns as compared with normal. The most recent addition to characteristics in development of intimal thickening is the presence and, in some cases, proven increase of apoptosis. Whether apoptosis solely reflects cell removal or may also provide signals by, for example, local modulating factors remains to be investigated.

In this mini-review we will focus on the current knowledge about (1) SMC origin from various embryonic mesodermal progenitors and (2) phenotypic heterogeneity in neonatal and adult vessel walls in various animal models. We will combine these data and hypothesize on the origin of SMC heterogeneity.

Embryonic Smooth Muscle Origin

The vascular system in the embryo recruits its first essential building blocks, namely, endothelial cells, from the splanchnic mesoderm. It is evident that endothelial differentiation is promoted at the endodermal–mesenchymal interface, although a dorsal somatic mesoderm-derived population has also recently been described. As soon as the endothelial progenitors are initiated, they tend to become mobile and form a vascular network that shows great plasticity. The next phase in development is the acquisition of a media for the larger vessels, ie, the arteries and the veins.

There are several reasons to revisit the discussion about the splanchnic mesoderm as a sole or main contributor. First, it is generally assumed that the splanchnic mesoderm surrounding the endodermal–mesenchymal interface,15 although a dorsal somatic mesoderm-derived population has also recently been described. As soon as the endothelial progenitors are initiated, they tend to become mobile and form a vascular network that shows great plasticity. The next phase in development is the acquisition of a media for the larger vessels, ie, the arteries and the veins.

This does not imply that we do not have valuable cytoskeletal and contractile protein markers with which to follow SMC differentiation from early development to the mature vessel wall. In the range of the contractile markers, there is an increasing list found in various animal species, including man, such as SM22, smoothelin, and smooth muscle (SM) myosin. It is important to note that they all are expressed in time after SM a-actin -actin turns out to be the most useful early marker that, in vivo, is not expressed in the lining endothelial cells during embryonic development. The value of the cytoskeletal markers desmin, vinculin, and meta-vinculin as SMC differentiation indicators is still being investigated. Study of a human fetal myofibroblast cell line indicates a specific role
for the splice variant meta-vinculin for carrying a contractile
inducing element. A further reason to revisit the mesodermal origin of SMC
is the evidence that has turned up from in vivo experiments in
which it was shown that the first layers of SM $\alpha$-actin–
expressing cells around the endothelial cell-lined tubes trans-
differentiate from the endothelium. It should be studied
whether this mechanism is specific for the dorsal aorta, where
it was first detected (Figure 1), or whether it is a more general
phenomenon, not only in normal development of the vessel
wall, but also as a recurring process in development of intimal
thickening in vessel wall disease.

Next to endothelial and splanchnic mesodermal origin,
there is evidence for a number of other sources, which are in
part also splanchnic mesoderm derived, such as the origin of
the coronary SMC. These cells arise from the epicardial
lining in a transformation process that also produces the
adventitial cells of the coronary vessel wall (Figure 2a and
2b), as shown in chicken–quail chimeras by our group. Similar
findings have recently been reported with retroviral
reporter gene tracing of epicardium. In the development of
the coronary vasculature, we have no evidence as yet that the
endothelial cells contribute to the SMC population by
transdifferentiation.

A final proven SMC source, previously attributed to
endothelial cell–SMC transformation, is that of neuroectoder-
mal origin, namely, the mesectoderm of the neural crest.
Careful mapping in chicken–quail chimeras and with retroviral
reporter gene transfer experiments shows that neural crest cells can differentiate into SMC in the thorax,
head, and neck arteries. In part this has been described
earlier. What is new is that neural crest cells are also
present in the wall of the cardinal veins.

Neural crest mapping shows that there are marked bound-
daries (Figure 3) between the vessels that do and do not contain

Figure 1. Transverse section of the dorsal aorta of a stage 17
chick embryo, double stained for $\alpha$-SM actin HHF35 (brown)
and X-galactosidase (blue). The endothelial cells (EC) were
labeled with the retroviral vector containing Lac Z at stage 14.
Note that a cell that once participated in the endothelial lining
(as determined by blue staining) is now in a deeper position and
presents with $\alpha$-SM actin (brown), indicative of endothelial-
SMC transformation. Scale bar is 50 $\mu$m.

Figure 2. Sections through the coronary artery (CA) connection
to the aorta (AO) in a stage 37 chicken–quail chimera. The epi-
cardium is derived from the quail with the chick as a host. a.
The all-quail specific marker QCPN shows that both media and
adventitial cells (brown dots) are derived from epicardium. b,
Media (M) of the CA is positive for SM $\alpha$-actin (brown). Scale
bar is 100 $\mu$m.

Figure 3. SMC derived from the neural crest are marked with
the retroviral LacZ construct by flushing the neural groove at
stage 9 to 10. Note the sharp boundary in this heart-lung speci-
men between neural crest and non-neural crest cells (arrow) at
the arterial pole of the heart. This heart-lung specimen is from a
chick embryo that was sacrificed at stage 39. A indicates
atrium; and V, ventricle.
nerves or neural crest cells.33-36 Non-neural crest arteries are the pulmonary arteries, the coronary arteries, and the subclavian arteries. A summary of known sources is presented in the Table.

In studying the origin of the vascular SMC there is clear evidence that the SMC and the fibroblast have many features in common regarding their origin. This is supported by our own studies on neural crest homing33 and on the origin of SMC and fibroblasts of the coronary vasculature (Figure 2a and 2b), as well as from data on endothelial SMC transformation (Figure 1), in which the adventitia of these vessels has a similar embryonic contribution as the media, suggesting a common origin of fibroblasts and SMC.

Summarizing the data on SMC origin, we can state that SMC were experimentally proven to originate from the neuroectoderm (neural crest) and from multiple mesodermal sources that have undergone differentiation to some extent (endothelium, epicardium). In addition, alleged media acquisition from the still undifferentiated splanchnic mesoderm undoubtedly plays a pivotal role, but was not yet verified by lineage studies.

### SMC Heterogeneity In Vivo and In Vitro

Heterogeneity of SMC is a well-known phenomenon. The interpretation, however, of the morphologic phenotype as well as the functional implications of the expression of differentiation markers is subject to much debate. The data are derived from in vivo or in vitro studies. The material used varies from adult to neonatal to fetal vessels and cells from different animal species. The origin of the cultured cells can be from intima, inner or outer media, or the adventitia in both normal and diseased vessels.

The data from in vitro studies are largely reflected in in vivo studies. Proceeding from data in early fetal development, it has been shown that the SMC start to express SM α-actin7 followed by 1E12,20 an actin marker, and smoothelin.21 Relative late differentiation markers are the already mentioned markers, such as SM22, calponin, h-caldesmon,7 and SM myosin.8 These are upregulated until the expression level of the mature vessel wall is reached.9,11 During this developmental period, there are also a number of markers that are downregulated, such as cytokeratin37 and certain fibronectin splice variants.11,29,38 When the various vessel wall layers are taken into account, the SMC at the intimal side of the vessel wall are less differentiated as compared with the middle and outer media.11,30

On development of intimal thickening, either as a physiological process as in the ductus arteriosus11,40,41 or as arteriosclerotic processes42 and restenosis,3 the reexpression of fetal genes is generally reported.

In general, the SMC are oriented circumferentially in the outer media, but close to the lumen they appear in a more random fashion. A few vessels show clusters of cells with a specific differentiation pattern. In the bovine pulmonary arteries, clusters of cells express meta-vinculin in a stable fashion during maturation,8 allowing for the differentiation of a subpopulation of contractile cells. A possibly different phenomenon is seen in the ductus arteriosus, in which during the closing process and the concomitant reexpression of fetal genes, clusters of cytokeratin-positive cells appear after general fetal cytokeratin expression was already lost.37

Combining the data from the in vitro studies, it can be concluded that from a morphologic point of view we can differentiate between at least 2 SMC phenotypes, namely the epithelioid and the spindle-shaped44-43 cell. To these can be added the thin elongated cell and the senescent type.3 These morphologically identified types most probably coincide with the more functional in vivo classification of contractile (resembling the spindle-shaped) and synthetic (resembling the epithelioid-shaped) cell types.44 The synthetic cell type is in some studies referred to as a nonmuscle phenotype because it lacks the contractile differentiation markers.27 The distinction of the SMC into a pup and a μ type45,46 brings the pup within the epithelioid range and the μ within the spindle-shaped range. Comparing fetal, neonatal, and adult data, phenotypic differences are already evident from early development onward but tend to become more prominent. The phenotypes are particularly obvious in the adult vessel wall, with a preference but not a selective presence of the synthetic type in the intima and the contractile cell in the media. Culture studies show that these phenotypic characteristics are relatively stable, at least when a confluent monolayer is achieved.44 The spindle-shaped phenotype has been described as being able to alter its phenotype to an epithelioid phenotype when culture conditions are suboptimal.44 Whether this change in phenotype can also be observed in vivo remains to be proven. The studies of Bochaton-Piallat et al46 show that both the intimal layer and the media contain a mixture of the various phenotypes. The ultimate fate of the SMC phenotypes is not identical, because the epithelioid/synthetic type is more prone to apoptosis both in vitro and in vivo44 in physiological31 and in pathological intimal thickening.3,47

The morphologic phenotype does correlate in general with the expression of certain differentiation markers. The spindle-shaped cells, accounting for the majority of the so-called contractile SMC, express differentiation markers such as SM22, calponin, and h-caldesmon,7 which relate to the contraction capacity of the cells. The expression of these markers is preceded by some fibronectin differentiation markers, such as fibronectin splice variants, troponin, and vinculin. These markers are more obvious in the fetal SMC phenotype, and some are lost in the neonatal and adult cell culture studies. A reexpression of these markers is seen in development of intimal thickening,42,45 being indicative of a dedifferentiation of the SMC. A similar phenomenon is seen

---

### Table: Embryonic Origin of SMC in the Thoracic Arteries

<table>
<thead>
<tr>
<th>Arteries</th>
<th>Mesoderm</th>
<th>EC</th>
<th>NC</th>
<th>EPDC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascending aorta</td>
<td>?</td>
<td>?</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Aortic arch</td>
<td>?</td>
<td>?</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Descending aorta</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Subclavian artery</td>
<td>+</td>
<td>?</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Pulmonary trunk</td>
<td>?</td>
<td>?</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Pulmonary artery</td>
<td>?</td>
<td>?</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Ductus arteriosus</td>
<td>?</td>
<td>?</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Coronary artery</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
</tbody>
</table>

EC indicates endothelial cells; NC, neural crest; EPDC, epicardium-derived cells; ?, not studied/not known; +, present or proven; and −, absent or not found.
in cytolytic necrosis of the media, as seen in the ductus arteriosus during the closing process.\textsuperscript{11} The reexpression of fetal markers is accompanied by an increase in apoptosis.\textsuperscript{3,11} The fetal cytoskeletal differentiation markers seem to persist in the so-called myofibroblast cell lineage.\textsuperscript{26} The role of this intriguing cell type either as an adventitial cell or as a smooth muscle stem cell remains to be investigated. In whole vessel wall organ cultures it has been shown that these myofibroblasts migrate from the adventitia to the luminal surface, where they transdifferentiate into an endothelial-like cell lining the lumen, as well as into a synthetic phenotype SMC in the intimal thickening.\textsuperscript{48} In this respect the so-called dendritic cells\textsuperscript{49,50} also need further investigation as their origin and role are still obscure.

The possible presence of a quiescent population of SMC that serves as a progenitor for the synthetic cell type will be discussed in the paragraph on relation of origin and phenotype.

In general, SMC heterogeneity is eminent both in in vitro and in vivo studies. For the latter experiments, the range of phenotypic expression is broader, as expected. This might bear relevance for the understanding of pathologic conditions.

**Elastic Versus Muscular Differentiation**

A major point that has not been solved until now concerns regulation of differentiation of vessels into an elastic or a muscular phenotype.\textsuperscript{27} The blood vessel architecture is well adjusted to the demands of maintaining blood pressure and propelling blood to the various organs, but the how and why of differentiation of a particular vessel into, for example, an elastic ascending aorta, a muscular coronary artery, and a muscular ductus arteriosus with intimal thickening remains elusive. In this matter, early regulatory genes, such as hox genes that determine pharyngeal arch patterning\textsuperscript{51} or the paired-related homeobox genes Prx1 and Prx2\textsuperscript{52,53} that have a vessel wall expression, do not easily explain vessel wall patterning. The ductus arteriosus remains an intriguing vessel as it shows a peculiar pattern for many molecules (Figure 4). There is a very high expression of angiotensin II (Blankesteijn WM, Gittenberger-de Groot et al, unpublished data, research started in 1996 and still in progress) in the fetal ductus as well as a high ductus-restricted expression of Prx2.\textsuperscript{52,53} The outer media of the ductus shows retinoic acid-responsive signaling,\textsuperscript{54} which also gives no further clue as to its meaning except that it correlates with a more differentiated SMC population.\textsuperscript{31,39}

In conclusion, the regulation of the vessel wall differentiation pattern and its boundaries are not well understood. It is too simple to hold hemodynamic parameters solely responsible. Most probably, regulating genes, cell origin, and functional demands interact in the resulting vessel wall phenotype.

**Correlation of Origin and Heterogeneity of SMC**

The specific characteristics of the SMC in intimal thickening in atherosclerosis have put several investigators on an interesting conceptual course of allowing these cells to be monoclonal in origin, deriving possibly from a single source.\textsuperscript{55} The origin of such a stem cell could in principle reside in both mesoderm and mesectoderm, ie, the sources of SMC. As intimal thickening as a pathologic process takes place in many vessels that may or may not contain a neural crest SMC-derived component (Table), neural crest cells do not seem to supply a specific SMC phenotype or more specifically an SM stem cell. There are indications, however, that neural crest-derived SMC do have a modulating effect on the formation of the elastic wall structure.\textsuperscript{27,56} but this is a relatively late phenomenon. Neural crest-derived SMC behave differently from mesoderm-derived cells, which can be deduced from in vitro culture studies that show a different reaction pattern of SMC to transforming growth factor-\(\beta\).\textsuperscript{57,58} The problem in the analysis of this in vitro study is that it does not reflect the muscular and nonmuscular SMC phenotypes that have been shown by Bergwerff et al\textsuperscript{13} (Figure 5a and 5b) to both have a neural crest background.

A second stem cell candidate may be the endothelial cell\textsuperscript{17} that was recently described to transdifferentiate into an SMC. Tracing of these cells, by means of a retrovirally introduced reporter gene, shows that the endothelial cells end up in the media (Figure 1) and even migrate into the adventitia. Here they coexpress the reporter gene and the SMC markers caldesmon and calponin (not shown). It remains to be investigated whether in normal development all atherosclerosis-prone vessels have acquired an endothelial cell-derived SMC contribution. In this respect also the adventitial fibroblast may play a role, although this cell also has a variable embryonic background.

In conclusion, we can state that SMC have a variable embryonic origin throughout the body. In relation to this origin, a variation in differentiation can be seen but not to the level of altering the basic elastic and muscular arterial patterning.

---

**Figure 4.** Cross-section through the continuity of the pulmonary trunk, the sixth (pulmonary) pharyngeal arch artery (PA), and the ductus arteriosus (DA) of a stage 37 chick embryo. All arteries have been shown to receive neural crest cells as an SMC source. Note the difference in lamellar organization of the alternating positive and negative SM \(\alpha\)-actin layers in the pulmonary artery as compared with the diffuse staining in the inner media of the DA. Scale bar is 100 \(\mu\)m.
Conclusions

During development and with increasing maturation, SMC acquire 2 to 4 morphologic phenotypes\textsuperscript{1,8,44} that have their preferential sites within the vessel wall layers. In experimental in vitro conditions these cells can be forced to change their phenotype but it is also possible with careful selection of the in vitro culture conditions to maintain a stable phenotype for many passages. In the in vivo situation these phenotypes can be traced to preferential sites within the vessel wall. There is evidence that the SMC phenotypes retain their differentiation state under normal circumstances. In disease it remains to be proven whether there is really a phenotypic modulation of one cell type into another, or whether the stem cell populations provide for the required phenotype. In vivo vessel wall studies with reporter gene tracing in the chicken model will be relevant to solve this problem. If transgenic mice can be devised that retain the endothelial cell and SMC promotor activity (eg, based on the Cre-loxP recombination system), phenotypic differentiation changes can be monitored. There is as yet no convincing evidence that the SMC heterogeneity has an origin-based background.

References


Smooth Muscle Cell Origin and Its Relation to Heterogeneity in Development and Disease
Adriana C. Gittenberger-de Groot, Marco C. DeRuiter, Maarten Bergwerff and Robert E. Poelmann

doi: 10.1161/01.ATV.19.7.1589
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1999 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/19/7/1589

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org//subscriptions/