Review

Origins of Aortic Coarctation: A Vascular Smooth Muscle Compartment Boundary Model

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Origins of Aortic Coarctation: A Vascular Smooth Muscle Compartment Boundary Model

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Abstract: Compartment boundaries divide the embryo into segments with distinct fates and functions. In the vascular system, compartment boundaries organize endothelial cells into arteries, capillaries, and veins that are the fundamental units of a circulatory network. For vascular smooth muscle cells (SMCs) such boundaries produce mosaic patterns of investment based on embryonic origins with important implications for the non-uniform distribution of vascular disease later in life. Morphogenesis of blood vessels requires vascular cell movements within compartments as highly-sensitive responses to changes in fluid flow shear stress and wall strain. These movements underlie remodeling of primitive plexuses, expansion of lumen diameters, regression of unused vessels, and building multilayered artery walls. Although loss of endothelial compartment boundaries can produce arterial-venous malformations, little is known about the consequences of mislocalization or failure to form SMC origin-specific boundaries during vascular development. We propose that the failure to establish a normal compartment boundary between cardiac neural crest-derived SMCs of the 6th pharyngeal arch artery (future ductus arteriosus) and paraxial mesoderm-derived SMCs of the dorsal aorta in mid-gestation embryos leads to aortic coarctation observed at birth. This model raises new questions about the effects of fluid flow dynamics on SMC investment and the formation of SMC compartment borders during pharyngeal arch artery remodeling and vascular development.

Graphic Abstract:

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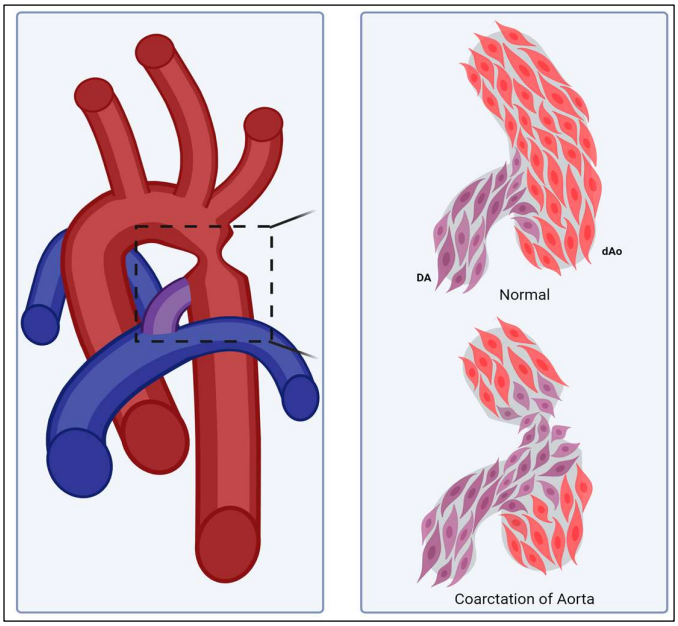

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Introduction

Coarctation of the aorta (CoA) is a form of congenital heart disease that is characterized by a structural narrowing of the aorta that restricts blood flow and thereby impairs normal growth and development of the newborn (**Figure 1**). Blood flow impairment is the result of a constriction of the descending thoracic aorta (AO) usually near the ductal ostia and is thought to be due to vascular smooth muscle cells (SMCs) from the ductus arteriosus (DA) mislocalized within the wall of the AO. CoA is one of the most frequent presentations of congenital heart disease (CHD), and the most common congenital defect of the aorta affecting 1 per 2,500 live births [1,2]. First diagnosed over two hundred years ago, CoA has been surgically repaired for the last 50 years yet we have little understanding of the underlying pathobiology. Genome sequencing of CoA and other CHD patient cohorts over the last decade has identified a handful of associated genes, but this effort has failed to establish a unifying mechanism for CoA [3–8].

Figure 1. CT image of a constriction site (arrow) in the region where the DA joins the descending AO in a neonatal patient with CoA.

Development of the Ductus Arteriosus

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In searching for the origins of CoA, one must look to the formation and remodeling of the pharyngeal arch arteries (PAAs) in midgestation embryos. This is because the DA arises from flow-sensitive remodeling of the 6th PAA in vascular development [9,10] (**Figure 2**) and because CoA can be detected by echocardiography in utero [11,12]. Although anatomically continuous with the aortic media, DA-SMCs in the ductal media do not normally extend beyond their junction with the descending thoracic aorta [9,13,14]. As the 6th PAA begins to assemble its complement of SMCs, two types of SMC progenitor cells are present in the pharyngeal arch complex: cardiac neural crest (CNC) and second heart field (SHF) [15]. Endothelial cells of both the 4th arch and 6th arch arteries are derived from SHF progenitors [16]. However, fate mapping studies have shown that medial SMCs of the 6th PAA originate exclusively from cardiac neural crest cells (CNCs) [17]. By contrast, medial SMCs in the dorsal aorta at the level of the 6th PAA junction arise from paraxial mesoderm (PM) [18]. Therefore, a border is formed at the interface between CNC-derived SMCs in the 6th PAA and PM-derived SMCs in the dorsal aorta. Elzenga et al. described the normal DA-AO junction as a "fish tail-like" insertion of DA-SMCs within the AO media involving up to one-third of the AO circumference in a newborn infant [19] (**Figure 3**). The formation of an insertion junction, therefore, means that the DA-SMC interface with AO-SMCs does not exactly correspond to the anatomical landmark of a DA (or later, LA) connection to the AO. Nevertheless, the juxtaposition of CNC-derived DA-SMCs and PM-derived AO-SMCs appears to result in a SMC origin-dependent border that, in effect, acts as a sorting mechanism keeping DA-SMCs confined to the limits of the ductal insertion and maintaining AO-SMCs in a separate anatomical compartment [19]. The necessity of a sorting mechanism becomes evident when the baby takes its first breath at birth. Loss of placental-derived prostaglandins together with an increase in arterial oxygen tension resulting from nascent blood flow to the lungs initiates closure of the DA, a critical event required for adequate perfusion of the now functioning lungs [14,20,21]. The directly adjacent AO-SMCs in the aortic media exhibit none of these oxygen-dependent closure activities. While the DA closes, the AO media continues to increase circumferential growth in proportion to increasing cardiac output required for postnatal growth of the neonate. The mechanisms that specify 6th arch SMCs to acquire a unique ductal SMC identity while, in close proximity (**Figure 2, note the proximity of IV to VI PAAs to each other**), dorsal aorta SMCs acquire an entirely different identity and developmental trajectory remain an intriguing mystery yet to be solved.

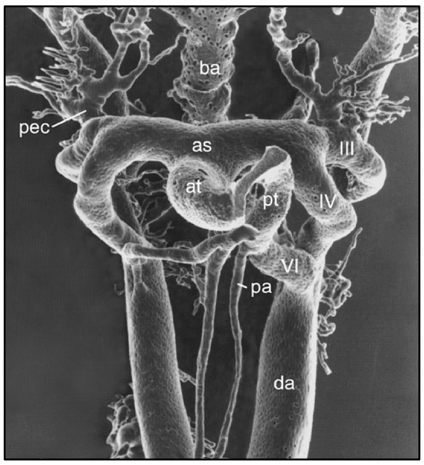

Figure 2. Vascular cast of pharyngeal arch arteries (PAAs) in an E12.0 mouse embryo visualized by scanning electron microscopy. Note the close proximity of PAAs IV and VI during the period of SMC investment and flow-dependent remodeling of the PAA complex. Reprinted with permission from [9].

Compartment Boundaries in Embryonic Development

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Formation of tissue boundaries that divide the embryo into compartments with distinct cell fates is one of the most fundamental processes in development [22–27]. For example, compartment boundaries separate adjacent rhombomeres during hindbrain development [28–30], somites during axial patterning [31–33], septation of the cardiac interventricular septum [34,35], pattern dorsal-ventral domains of the limb bud [36,37], and delineate imaginal discs during development and regeneration in *Drosophila* [38,39]. Compartment boundaries are formed when cells with different identities encounter each other [34,35,38,40]. A lack of mixing at such borders is a direct result of compartment-specific transcriptomes and maintenance of different cell identities and cell fate potentials. Studies of the *Drosophila* embryo have provided a conceptual framework for morphogenesis that includes segmentation, acquisition of cell identity, compartmentation, and pattern formation [40,41]. An important principle that has emerged is that the early embryo is organized into lineage blocks called compartments [38]. Two different lineage blocks that form adjacent to each other are separated by a compartment border that is maintained as a function of different cell identities on either side of the border [38]. If an imaginal disc compartment border is disrupted, for example, then the two compartments regenerate the missing cells, reestablish compartment-specific cell identity, and reorganize the border between them [42]. What mechanisms actually produce compartment borders have been the subject of much investigation [40]. These studies have produced three general models: the differential cell adhesion model [43–45], the actin-myosin cortex contraction model [40,46], and the contact repulsive model focused on heterotypic cell-cell contacts [25,27].

Compartment Boundaries in Vascular Development

In the vascular system, three types of developmental borders have been identified. One is the boundary separating arterial and venous endothelial cells [47–49] or arterial and venous SMCs [48,50,51] marked by guidance molecules that mediate contact repulsive signaling between adjacent heterotypic cells. A second type of border separates aortic root endothelium of second heart field origin [16] from coronary artery endothelium of sinus venosus origin [52]. This represents an artery-artery EC boundary and, once established, was found to be stable out to at least postnatal day 28 in the mouse [52]. A third type of compartment boundary separates SMCs of different developmental origins in a common artery wall [53–59]. Although multiple descriptions of these SMC lineage-specific compartments have been made in the studies cited above and elsewhere, little is known about SMC compartment borders themselves, the molecules & mechanisms that produce these borders, the compartmentalization of SMC functions they maintain, or the consequences of a loss of border function in vascular dysmorphogenesis and disease. Drawing clues from compartment boundary studies in *Drosophila* and other model organisms [25,60,61], we will discuss these questions with a particular focus on the DA-SMC junction with AO-SMCs in the proximal descending thoracic aorta.

Repulsive Guidance Molecule Signaling

Chemo- or contact-repulsive signaling is one of the key border-forming mechanisms employed in embryonic development [51,62,63]. Contact-repulsive guidance molecules are expressed by CNC-derived SMC progenitors enabling them to precisely navigate to the 6th PAA during vascular development [51,64–70]. Likewise, migration of paraxial mesoderm-derived AO-SMC progenitors to the dorsal aorta is also guided by repulsive signaling [31]. During neural crest cell migration in Xenopus embryos, the homophilic calcium-dependent cell-cell adhesion molecule cadherin-11 was found to be necessary for contact inhibition of movement [71]. This may reflect the cooperative activity of cadherin 11-dependent cell-cell adhesion to form close contacts between adjacent cells so that contact-repulsive mechanisms are efficiently engaged by those cells [72]. That engagement may initiate the contact-mediated collapse of cell protrusions oriented in the direction of migration [73]. A similar role for N-cadherin in CNC migration has been reported [74]. Upon contact of DA-SMCs with AO-SMCs, we suggest that engagement of contact-repulsive guidance molecules stops

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migration of both types of vascular SMCs, initiates border-dependent cytoskeletal reorganization [75], and creates a barrier that prevents mixing of DA-SMCs and AO-SMCs. The abruptness of the DA-AO SMC border [76] (**Figure 3**) implies a boundary-forming mechanism similar to that which separates arterial and venous endothelial cells in early vascular development [47,49,77,78]. The report of a Semaphorin-3D (*SEMA3D)* loss of function mutation in human CoA is consistent with this hypothesis [6].

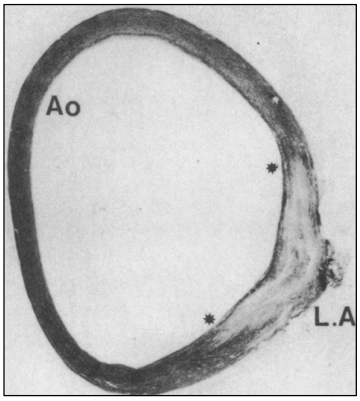

Figure 3. "Fish tail-like" insertion junction (between the asterisks) of human ductus arteriosus SMCs (LA) into the AO media (reprinted with permission from [19]. Note the absence of organized elastic fibers in the ductal insertion. LA – ligamentum arteriosum.

It is instructive to consider SMC border formation at another site in the developing vascular system. Lineage- and fate-mapping studies in mouse embryos have shown that SMCs in the aortic root are derived from progenitor cells of either cardiac neural crest or second heart field origin [79]. Sawada et al. showed that these two SMC origins reside in distinct spatial domains in the ascending thoracic aorta with little or no detectable mixing [56]. Cardiac neural crest-derived SMC progenitors migrate into the pharyngeal arch complex and reach the 4th arch artery wall earlier than second heart field derived SMC progenitors and form the first few inner layers of the aortic root and ascending aorta [79]. Slightly delayed in time, second heart field-derived cells also migrate to the developing aorta and constitute the major SMC type in the aortic root, and form the outer medial layers of the ascending aorta [53,55,56,79]. These two types of aortic SMCs express similar levels of SMC contractile marker genes and cytoskeletal genes but they retain SMC lineage-specific differences in TGF-beta receptor-dependent growth and transcriptional responses [80,81], baseline gene expression patterns [59,82], and accessible chromatin sites identified by ATAC-seq analysis [83]. Likewise, SMCs from different embryonic origins express different levels of extracellular matrix genes in vivo [84] and in vitro [59].

Remodeling of the Pharyngeal Arch Artery Complex

Endothelial cells: Developing blood vessels are highly sensitive to blood flow variations [15,85–87,92,95]. Studies in multiple species have shown that changes in blood flow rates are major morphogenic forces acting on endothelial cells directing their growth, migration, and cell-cell interactions [10,85–91]. Fluid shear stress on the endothelial cell surface regulates the assembly of endothelial cell junctions, cytoskeletal organization, polarity, and arterial-venous identity [85,92–95]. In particular, the complex remodeling of the PAAs is, in large part, determined by developmental changes in cardiac function, blood flow dynamics, and endothelial shear stress forces [10,89,96]. Using fluorescent dye injection, pulsed Doppler velocity recordings, and micro-CT scans, Wang et al. showed that a shift occurs in flow velocity and wall shear stress (WSS) in the PAA complex between stages HH18 and HH24 in the chick embryo [97]. That shift is from a higher WSS in PAA-3 at HH18 to a higher WSS in PAA-4 at HH24 [87,97–99]. These developmental shifts in flow velocity and WSS

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are recorded as corresponding diameter changes in the respective PAAs during the remodeling period [87,97,99].

There is considerable evidence that defects in heart or valve development and resultant changes in blood flow rate, flow distribution, and endothelial shear stress responses during cardiovascular development produce congenital heart defects [98–100]. There is also evidence that both endothelial cells and SMCs are surprisingly motile within the vessel wall during the early stages of vascular development [91,101]. It therefore follows that the positions of the 6th PAA ostia with the dorsal aorta and pulmonary artery, the investment of this vessel with SMCs, or the SMC origin-specific border between 6th PAA SMCs and dorsal aorta (AO) SMCs may be sensitive to disturbances in normal blood flow dynamics in this critical period of PAA remodeling.

Smooth muscle cells: For example, the murine left pulmonary artery (PA) at birth consists of an inner layer of endothelial cells, two layers of SMCs and an outer layer of adventitia [101]. The first layer of PA-SMCs emerges from PDGFRβ-positive mesenchyme surrounding the developing PA wall at around E11.5. As SMC differentiation markers appear in the first layer of SMCs, expression of PDGFRβ is down-regulated in these cells [101]. The second layer of PA-SMCs arises from two sources. The major fraction originates from surrounding PDGFRβ-positive mesenchyme in a sequence much like the first layer. Examination of nuclear morphology and the orientation of SMC mitotic spindles during PA wall formation showed that during E11.5 to E13.5, the axis of division of SMCs in the first layer was predominantly longitudinal (>75%) thus contributing daughter cells to growth in length of the PA. At E14.5, however, the axis of division shifts so that the majority of first layer SMCs divide circumferentially while cells in the second layer of media and in the adventitia continue to divide in the longitudinal orientation. Therefore, circumferential orientation of division of first layer SMCs contributes daughter cells to expand the second layer. Using a single low dose of tamoxifen in *Myh11CreERT2; mTmG* mice administered at E11.5 to label individual SMCs, the position of clones originating from inner layer SMCs could be mapped in the PA wall from E13.5 to E18.5. These experiments showed that individual cells within a clone often dispersed widely from each other, intermixing with unlabeled cells, and populating both first layer and second layer SMCs. While migrating extensively within the media both longitudinally and circumferentially, labeled SMCs did not enter the adventitia or the intima. These results illustrate two important principles about early vascular development. The first is that cells within nascent blood vessel walls are not stationary, but exhibit considerable motility within the developing vessel or primitive vascular network. The second is that barriers, or borders, exist that define compartments into which entry by cells from outside the compartment is prevented. As described in a previous section, compartments and the borders that define them are fundamental to tissue morphogenesis and it is no surprise to also find them in developing blood vessels.

The Role of Hemodynamics in PAA Remodeling

The *shrunken head* (*shru*) mutation was identified in a forward genetic screen in mice and found to be localized within the *titin* gene [10]. *Shru* produced a hypomorphic allele and embryos were defective in development of myocardial cells as revealed by greatly reduced numbers of myofibrils in these cells, reduced cardiac contractile activity, and pronounced delay in the onset of circulation through the vascular system. *Shru* mutants had little or no blood flow from E8.5 to E9.5 and then exhibited a weak initiation of blood flow from E9.5 to embryonic death at E11.5 [10]. A variety of embryonic and extra-embryonic vascular defects were observed in *shru* mutant embryos including defective endothelial cell-cell junctions, abnormal endothelial cell protrusions, intermittent lack of lumen formation, extensive areas of hypoxia, and increased *VEGFA* gene expression. As titin is expressed in cardiac myocytes but not in endothelial cells, the vascular defects in *shru* embryos are most likely indirect and due to greatly reduced or absent blood flow through the developing vascular system during angiogenesis, plexus reorganization, and PAA remodeling stages. The authors conclude that fluid biomechanical forces produced from the very onset of cardiac contractions are required to orchestrate multiple aspects of endothelial structure and function as well as vascular

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network formation and remodeling [10]. It will be of interest to learn if the formation or position of SMC origin-specific borders is disrupted in *shru* embryos.

The outcome of PAA remodeling is also a reflection of the overall control of left-right asymmetry (laterality) during embryonic development [98,102,103]. Control of laterality for internal organs is a function of nodal signaling [102]. Nodal induces the expression of PITX2, a transcription factor that controls left-right asymmetry in cardiac development and aortic arch remodeling [103,104]. Asymmetric expression of *Pitx2* is controlled by the asymmetric enhancer element (ASE) in the *Pitx2* locus [102,105]. Mice lacking the ASE develop right isomerism, cardiac developmental defects, and randomized laterality of PAAs [105]. While asymmetry of 6th PAA remodeling is randomized in mice lacking the ASE, the 4th PAA was not affected [98]. Laterality of the aortic arch is also randomized in these mice and is dependent upon which side of the paired 6th PAAs undergoes regression. In wild-type embryos, echocardiography studies showed that at E11.5 blood flow through the left dorsal aorta was similar to that in the right dorsal aorta, whereas by E12.0 blood flow was significantly higher in the left compared to the right-side dorsal aorta [106]. Surprisingly, *Pitx2* is not expressed in or near the 6th PAA or the dorsal aorta. Rather, it is strongly expressed in the second heart field, the myocardium, and the left wall of the outflow tract [98,103]. In normal heart development, formation of the spiral septum of the outflow tract aligns the ventricles with the great arteries and rotation of the arterial pole produces changes in the right 6th arch artery structure that reduces blood flow and favors regression of the right side [107]. In ASE-deficient mice, defects in outflow tract morphogenesis result in altered blood flow distribution to the right and left sides of the 6th PAA leading to randomized laterality of the 6th PAA [98,99]. These studies show how closely related rotational development of the outflow tract is to normal asymmetric remodeling of the 6th PAA and ultimate formation of the DA. As indicated above for *shru* embryos, it will be interesting to determine if the position of the SMC origin-dependent border is disrupted if blood flow distribution through the right versus left 6th PAAs is altered due to defects in cardiac valve or outflow tract development.

Role of Hemodynamics in Vascular Smooth Muscle Cell Investment

As discussed above, PAA remodeling is highly sensitive to changes in blood flow. Evidence from multiple experimental models shows that investment of nascent endothelial tubes with mural cells (SMCs and pericytes) is also a blood flow-responsive process [108–110]. The release of PDGFB, among other factors, by endothelial cells is stimulated by increased fluid shear stress acting on the endothelial cell surface [111,112]. The abluminal secretion of PDGFB [113] and the activation of nearby PDGFRβ-expressing SMC progenitor cells are key steps in the investment process [114–118]. The effects of PDGFRβ signaling involve stimulation of SMC progenitor cell chemotaxis [112], migration [111], and localized cell proliferation in vivo [114]. Further analysis suggests two models for the investment process: (a) *de novo* formation of SMCs from surrounding undifferentiated mesenchymal progenitor cells, or (b) migration of SMCs from a pre-existing pool of SMCs usually upstream of the site of investment [110,114–116,119–121]. One question that is not addressed by these previous studies is how SMC investment proceeds at specific sites in the vascular system where SMCs arising from two different embryonic lineages are involved. What cell dynamics occur when cardiac neural crest-derived SMCs and paraxial mesoderm- or second heart field-derived SMCs encounter each other during assembly of the vessel wall? In any case, investment models must account for the apparent lack of mixing at SMC origin-dependent borders [53,54,122].

Interactions Between Different Types of Vascular SMC Progenitors

Investment of PAAs with SMCs exhibits the ability to compensate for defects in the number of available CNC-derived SMC progenitor cells. Alexander et al., reported that in mice made Smad4-deficient in the CNC-lineage using Sox10iCre, most of the CNC-derived SMC progenitors in the pharyngeal arch mesenchyme were lost due to cell death [123]. In the absence of this dominant source of PAA SMCs, a non-CNC source of locally-available SMC progenitors was observed to rescue the

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investment process so that the 4th and 6th PAAs developed and remodeled normally [123]. Although not tested directly, it is reasonable to assume that this non-CNC source of SMC progenitors was second heart field-derived progenitor cells which are also present in the PA mesenchyme [123]. This finding suggests the possibility that CNC-derived SMC progenitors normally suppress the SMC fate of available non-CNC progenitors providing a glimpse of the normal cross-talk between SMCs of different embryonic origins at compartment borders. If investment itself is a blood flow-dependent process, then the communication between SMC progenitors as well as the final structure or position of SMC origin-dependent borders may also be influenced by blood flow dynamics.

A Smooth Muscle Compartment Boundary Model for CoA

We propose a vascular SMC compartment boundary model for the pathogenesis of CoA. This model suggests that CoA is the consequence of a failure to form a SMC origin-specific compartment border in its normal position at the ductal ostia (**Figure 4**). We propose that disturbed blood flow during remodeling of the 6th PAA, either due to direct changes (somatic genetic or epigenetic) in the 6th PAA cells themselves, or indirect hemodynamic effects due to altered cardiac contractile activity, cardiac valve structure or function, or outflow tract defects could lead to an altered position or absence of the DA-SMC junction with AO-SMCs at the ductal ostia thus allowing DA-SMCs to invade the aortic wall (**Figure 4**). This would explain the localization of a majority of CoA lesions to the ductal-aorta junction and the clinical observation that CoA can be detected by fetal echo before birth [11,12]. It would also explain the frequent co-occurrence of CoA with bicuspid aortic valve and other cardiac congenital defects that would disturb blood flow during PAA remodeling [1,10,98,124,125]. This model for the origin for CoA raises important new questions: (1) if the DA-AO compartment border is the consequence of an interface of SMCs with different identities, similar to other developmental borders, then what molecules and mechanisms are responsible for the specification of distinct identities of DA-SMCs vs AO-SMCs during vascular development? (2) what cell surface molecules and signaling pathways normally act to prevent mixing of DA-SMCs and AO-SMCs at the ductal-aorta interface? and (3) what mechanisms disrupt the expression, function, or localization of these border-forming molecules during the pathogenesis of CoA? Given that vascular SMC origins are far more diverse than just CNC and SHF, the answers to these questions and the molecular insights gained may provide a better understanding of vascular pathologies at other sites within the mosaic vascular system as a whole.

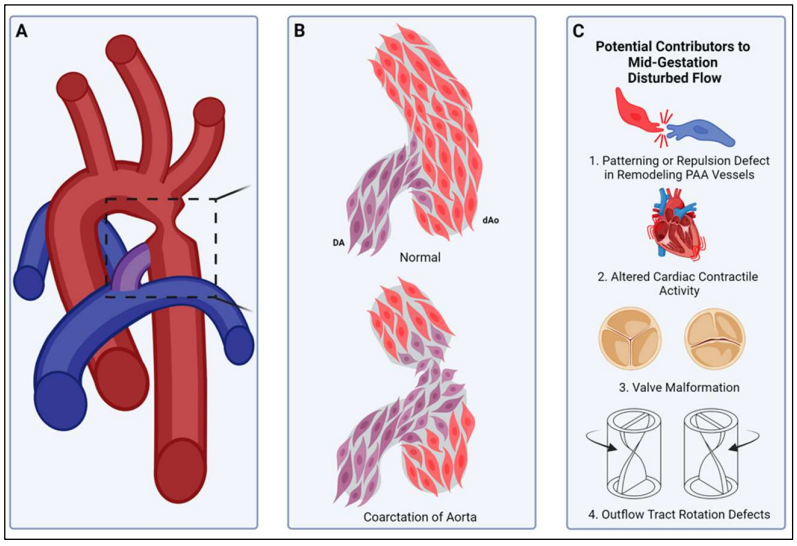

Figure 4. A smooth muscle compartment boundary model of CoA. (A) Illustration of a common site of aortic coarctation where the ductus arteriosus (purple) joins the descending thoracic aorta (red). Boxed area is

illustrated in more detail in panel B. (B, top) Normally, cardiac neural crest-derived DA-SMCs (DA, purple) and paraxial mesoderm-derived descending Ao-SMCs (dAo, red) fail to mix and a compartment boundary is formed at their interface (see insertion junction, Figure 3). (B, bottom) In coarctation of the aorta, loss of the compartment boundary results in DA-SMCs (DA, purple) invading the aortic media (dAo, red) producing an aortic constriction upon birth of the neonate. (C) Potential mechanisms for the pathogenesis of CoA. The normal DA-SMC interface with Ao-SMCs is established during SMC investment of pharyngeal arch artery (PAA) walls in mid-gestation (see, Figure 2). Remodeling of the paired PAAs is highly sensitive to changes in blood flow rate and flow distribution through the right and left sides of the PAAs [10,15,98,99]. Either disruptions in SMC cell-cell interactions that normally prevent mixing across SMC origin-dependent compartment boundaries (C1), or defects in cardiac contractile activity (C2), valve morphogenesis (C3), or outflow tract formation/rotation (C4), can alter the hemodynamic shear stress distribution during PAA remodeling. We propose that the formation of the normal DA-Ao SMC interface (B) is also a flow-sensitive process during PAA remodeling and disturbed patterns of SMC investment during PAA remodeling can produce CoA observed in the neonate.

Author Contributions: MWM and CLG wrote the article, GT contributed to preparation of the figures, and all authors made critical input and edits during preparation of the manuscript.

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Conflicts of Interest: There are no conflicts of interest to disclose.

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