© 2021. Published by The Company of Biologists Ltd | Development (2021) 148, dev197384. doi:10.1242/dev.197384

REVIEW

The origin and mechanisms of smooth muscle cell development in vertebrates

Michael Donadon and Massimo M. Santoro*

ABSTRACT

Smooth muscle cells (SMCs) represent a major structural and functional component of many organs during embryonic development and adulthood. These cells are a crucial component of vertebrate structure and physiology, and an updated overview of the developmental and functional process of smooth muscle during organogenesis is desirable. Here, we describe the developmental origin of SMCs within different tissues by comparing their specification and differentiation with other organs, including the cardiovascular, respiratory and intestinal systems. We then discuss the instructive roles of smooth muscle in the development of such organs through signaling and mechanical feedback mechanisms. By understanding SMC development, we hope to advance therapeutic approaches related to tissue regeneration and other smooth muscle-related diseases.

KEY WORDS: Smooth muscle function, Smooth muscle origin, Genetic, Lateral plate mesoderm, Smooth muscle differentiation, Transgenic

Introduction

Smooth muscle cells (SMCs) are specialized cells found mainly in the walls or in proximity of hollow organs, including the circulatory, respiratory and digestive systems (Halayko et al., 1996; McHugh, 1995; Owens et al., 2004; Sparrow and Lamb, 2003). Although not discussed in detail in this Review, SMCs are also present in reproductive system (oviduct and epididymis), urinary tracts, skin and eyes (Jahoda et al., 1991)

Depending on the organ of interest, SMCs can be found as single cells or they can be organized into layer-forming sheet of intertwined and elongated cells (Burnstock, 1970; Gabella, 1981). Unlike skeletal muscle, smooth muscle (SM) tissue does not have sarcomeres or striations, but does have actin and myosin proteins that both form and control their contractile apparatus. The contraction of SM is initiated by calcium ions (Ca^{2+}), which are released from sarcoplasmic reticulum and Ca^{2+}-calmodulin-myosin systems (Hill-Eubanks et al., 2011). SM tissues are not controlled voluntarily, and the regulation of contraction is dependent on hormones, on parasympathetic nerves through the autonomic nervous system (ANS) and on locally released signals, such as nitric oxide (NO). In addition, the epithelium and endothelium have been linked to the regulation of SM contraction (Box 1). Often, the stretching-relaxation control response (stretching the muscle to induce its contraction) can regulate SM contraction in certain visceral organs (Chevalier, 2018). It is this contraction and relaxation of SMCs that regulates the function of the organs in which they are found (Huycke et al., 2019).

Department of Biology, University of Padua, Via U. Bassi 58B, 35121 Padua, Italy.

*Author for correspondence (massimo.santoro@unipd.it)

M.M.S., 0000-0003-4605-5085

SMCs are identified through the expression of specific markers, such as α smooth muscle actin (αSMA/ACTA2), transgelin (TAGLN/SM22), smooth muscle-myosin heavy chain (SM-MHC/MYH11), caldesmon, serum response factor (SRF), myocardin and other signaling molecules (Table 1). Most of these markers are shared among SMCs; however, in principle, different combinations of them can be used to distinguish the different types of SM tissue present in circulatory (vascular SMCs and pericytes), respiratory (airway SMCs) and digestive (visceral/intestinal SMCs) systems (Table 1).

SMCs play crucial roles during organ development and morphogenesis, and provide different functions in adulthood depending on their location and organ distribution. In order to understand how a complete organ or tissue develops, it is crucial to reveal the mechanisms of SMC specification and differentiation (Badri et al., 2008; Majesky, 2007; McHugh, 1996; Nakano et al., 2011; Wang et al., 2015). Here, we describe the origin of SMC populations and the steps leading to SMC differentiation during the development of the cardiovascular system, airways and intestine. We describe how SMCs shape organogenesis and discuss how heterogeneities in the origins of SMCs could be used as an innovative therapeutic approach to treat SMC-related diseases.

Organization, function and developmental origin of smooth muscle cells

Although SMCs share similar characteristics, in vertebrates SMCs arise from a wide range of embryonic structures that not only vary between different organs, but also within the same tissue. In this section, we describe the organization and function of SMCs in different organs. We review some of the key fate-mapping experiments that have identified the prominent populations that give rise to SMCs in the cardiovascular system, the respiratory system and intestinal tract in zebrafish, chicken and mice (Jiang et al., 2000; Le Lièvre and Le Douarin, 1975; Santoro et al., 2009; Wasteson et al., 2008) (Fig. 1). Some of the specific genetic and molecular tools that have been used to investigate the developmental origin of SMCs are summarized in Table 2.

Smooth muscle cells in the cardiovascular system

SMCs that surround the circulatory system are called vascular mural cells and can be divided between vascular smooth muscle cells (vSMCs) and pericytes (Gaengel et al., 2009).

Vascular smooth muscle cells

vSMCs are the most well-studied population among SMCs because their function is essential for arterial physiology and pathology (Basatemur et al., 2019). vSMCs are organized into concentric layers within the walls of arteries and veins (Carmeliet, 2000; Herbert and Stainier, 2011), where they play an essential role in establishing and stabilizing blood vessels during embryonic development (Fig. 2). Furthermore, vSMCs act as crucial

Development (2021) 148, dev197384. doi:10.1242/dev.197384

Box 1. Epithelium and endothelium as regulators of smooth muscle contraction and function

Researchers have long been exploring the role of the endothelium and epithelium in regulating smooth muscle (SM) contraction. In the late 1970s, researchers began to collect evidence showing that the sole epithelium from several organs may influence SM contraction. These studies reached this conclusion by splitting the epithelium from the SM tissue in organ-bath experiments in rat, rabbit or guinea pig (Farmer et al., 1987; Whalley, 1978). In the lung, the ability of the mesenchymal cells to stretch over a surface is important in promoting aSMC differentiation (Yang et al., 2000). Here, it has been shown that sustained stretch-induced expression of SM proteins in undifferentiated mesenchymal cells accelerated the differentiation of mouse and human aSMCs. Later, more complex studies on mammals reinforced the notion that the epithelium and endothelium regulate SM contraction *in vivo* by releasing specific factors, such as acetylcholine, histamine or PGF2 (Gillman and Pennefather, 1998; Okpalaugo et al., 2002). Similarly, the endothelium is a source of molecules that either stimulate or inhibit the contraction of underlying SM cells, such as heparin/heparan sulfate, cytokines and growth factors, as well as nitric oxide and endothelins that influence vascular tone and SMC contraction/relaxation. Such factors may act together with other stimuli like proliferation, migration and differentiation (Forstermann and Sessa, 2012; Furchgott and Vanhoutte, 1989; Stratman et al., 2020; Wilson et al., 2016; Yanagisawa et al., 1988).

regulators of vessel wall assembly and vessel maturation, because they are involved in the production and maintenance of the basement membrane of blood vessels, as well as promoting endothelial cell (EC) quiescence and abolishing responsiveness to vascular endothelial growth factor (VEGF) (Korff et al., 2001; Wagenseil and Mecham, 2009). In the adult, they also provide structural and functional support to vessels to maintain correct blood pressure and tissue perfusion.

It is worth mentioning that the embryonic origins of vSMC progenitors are highly heterogeneous compared with other types of SMC. As we discuss below, vSMC progenitors derive from several lineages depending on the vascular region (Fig. 1). The first insights into the embryonic origin of vascular SM have been derived from a lineage map study for neural crest cells (NCCs) using chick-quail chimeras (Le Lièvre and Le Douarin, 1975). Through this lineage-tracing strategy, it has been possible to visualize that cranial and cardiac NCC migrate toward the pharyngeal arch complex and differentiate into vSMCs associated with the branchial arch arteries. These NCCs also provide SMC progenitors at the level of the right and left common carotid arteries, right subclavian artery (but not the left one), the ducts arteriosus, as well as the ascending aorta and aortic arch (Jiang et al., 2000; Kulesa and Fraser, 2000; Lumsden et al., 1991). Interestingly, substituting the cranial neural crest population with cells from trunk neural crest does not produce vSMCs in those anatomical regions, indicating that environmental cues alone are not sufficient to induce SMC differentiation and that intrinsic factors are necessary (Bookman et al., 1987; Kirby, 1989).

Conversely, it has been shown that vSMCs located at the level of the descending aorta (DA) derive from both splanchnic mesoderm and the somitic compartment, called the sclerotome (Jiang et al., 2000; Pouget et al., 2006; Wasteson et al., 2008; Wiegreffe et al., 2007) (Fig. 1). Lineage-tracing experiments have revealed two different populations of vSMCs in the DA during chick and mouse

Table 1. Smooth muscle cell markers and their functions

Marker	Function	vSMC	PC	aSMC	iSMC
α-Smooth muscle actin (αSMA/ACTA2)	Cytoskeletal element involved in fiber contraction	✓	x	✓	✓
γ-Smooth muscle actin (γSMA/ACTA2)	Cytoskeletal element involved in fiber contraction	✓	x	✓	✓
Smooth muscle myosin heavy chain (SMMHC/MYH11)	Smooth muscle myosin containing heavy chain and involved in fiber contraction	✓	x	✓	✓
Smooth muscle protein 22α (SM22α/transgelin)	Cytoskeletal element associated with actin filament and involved in fiber contraction	✓	x	✓	✓
Calpolin	Calcium-binding protein involved in the regulation of smooth muscle contraction	✓	x	✓	✓
Caldesmon	Calmodulin-binding protein involved in the regulation of smooth muscle contraction	✓	x	✓	✓
Serum response factor (SRF)	Transcriptional factor that interacts with different cues inducing SMC differentiation	✓	x	✓	✓
Myocardin	Transcriptional co-activator of serum response factor	✓	x	✓	✓
Vimentin	Intermediate filament highly expressed during embryonic development	✓	x	✓	✓
Desmin	Intermediate filament highly expressed after muscle differentiation and involved in the proper muscle structure	✓	x	✓	✓
Meta-vinculin	Vinculin-related protein involved in vinculin binding	✓	x	✓	✓
Smoothelin A	Acting-binding protein involved in the contraction of the muscle	✓	x	✓	✓
Smoothelin B	Acting-binding protein involved in the contraction of the muscle	✓	x	x	x
Neuron-glial antigen 2 (NG2)	Transmembrane proteoglycan involved in the proliferation and migration of the cell	✓	✓	x	x
Platelet-derived growth factor receptor β (PDGFRβ)	Receptor tyrosine kinase that acts like homo- or hetero-dimers involved in SMC proliferation, migration and differentiation	✓	✓	x	x
Regulator of G-protein signaling 5 (RGS5)	Regulator of G-protein signaling that inhibits signal transduction	✓	✓	x	x
Enhancer of zeste homolog 2 (EZH2)	Histone-lysine N-methyltransferase involved in the methylation and repression of target genes	x	x	✓	x
Leucine-rich repeat-containing G-protein coupled receptor 5/6 (LRG5/6)	Receptor involved in the Wnt signaling pathway and a marker for aSMC progenitors	x	x	✓	x
miR143/145	Controllers of SMC differentiation involved in the expression of SMC markers	✓	x	x	✓

aSMC, airway smooth muscle cell; iSMC, intestinal smooth muscle cell; miR, microRNA; PC, pericytes; SMC, smooth muscle cell; vSMC, vascular smooth muscle cell.

Development (2021) 148, dev197384. doi:10.1242/dev.197384

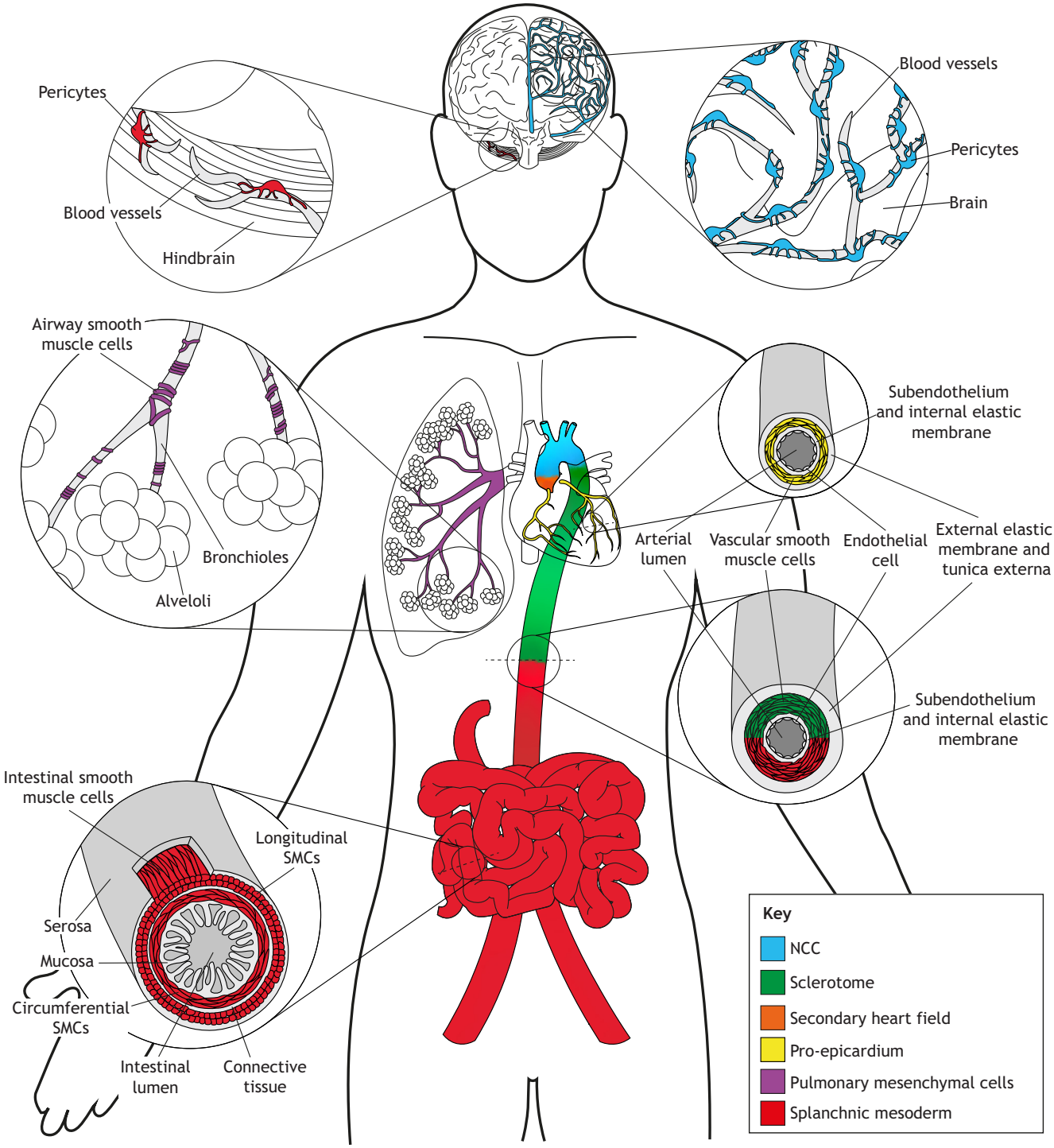

Fig. 1. Schematic representation of the developmental fate map for smooth muscle (SM) lineages. The different colors represent the different embryonic origins for the smooth muscle cells (SMCs). Vascular SMCs (vSMCs) are located at the level of arteries and veins as multi-layers of cells in the tunica media, and they show a wide spectrum of origins: the secondary heart field (orange) gives rise to vSMCs at the level of the base region of the aorta; and to neural crest cells (NCCs, blue) at the level of the right and left common carotid arteries, the right subclavian artery, the ductus arteriosus, the ascending aorta and aortic arch. Sclerotome (green) and splanchnic mesoderm (red) give rise to vSMC at the level of the descending aorta. A source of vSMCs for the coronary vasculature is the pro-epicardium (yellow). Pericytes are present at the level of the smaller capillaries (small arterioles and venules): they wrap around the endothelial cells but do not completely cover them. They extend primary and secondary processes along the surface of the endothelium. Pericytes have two main origins: NCCs (blue) give rise to pericytes located in the brain (except for pericytes located at the level of the hindbrain), while splanchnic mesoderm (red) gives rise to pericytes located along the trunk. Airway SMCs (aSMCs) surround the bronchial tree derived from the pulmonary mesenchyme (violet). Intestinal SMCs (iSMCs) are derived from the splanchnic mesoderm (red) and they form circumferential and longitudinal layers located in the inner and outer layer of the gut, respectively.

development (Wasteson et al., 2008; Wiegreffe et al., 2007, 2009): during early development, upon aortic fusion, splanchnic mesoderm-derived cells colonize the ventral regions of the DA and start to express transgelin, a marker of SMC differentiation. These cells are referred to as ‘primary SMCs’ (Wiegreffe et al., 2007). Soon after, sclerotome-derived cells invade and colonize the

Development (2021) 148, dev197384. doi:10.1242/dev.197384

Table 2. Tools to study smooth muscle cell origins in vertebrates

Tissue	SMC progenitor source	Vertebrate model	Target tissues	Tools	Techniques	Reference
Cardiovascular system	Early migratory NCCs	Mouse	Pharyngeal arch arteries, ascending aorta, right and left common carotid arteries, right subclavian artery, ductus arteriosus and aortic arch	*Wnt1-Cre* transgene and *R26R-LacZ* reporter allele	Cre/lox recombination	Jiang et al. (2000)
	Cardiac pericytes and vSMCs	Mouse	Coronary vasculature	*Pdgfrb-Cre* and *Rosa26-mT/mG*	Cre/lox recombination	Chen et al. (2016)
	Lateral plate mesoderm	Mouse	Descending aorta	*Hoxb6-Cre* transgenic and *ROSA26-LacZ* reporter allele	Cre/lox recombination	Wasteson et al. (2008)
	Lateral plate mesoderm	Zebrafish	vSMC	Transgelin antibodies, and *hand2* and *cloche* mutants	Immunofluorescence and ENU mutagenesis	Santoro et al. (2009)
	NCCs	Chick	Branchial arches	*In ovo* long-term time-lapse confocal microscopy	DiI labeling	Kulesa and Fraser (2000)
		Zebrafish	PC	*TgBAC(pdgfrb: Gal4FF); Tg(UAS: loxP-mC-loxP-mV)×Tg(sox10:Cre)*	Cre/lox recombination and lineage-tracing analysis	Ando et al. (2016)
	NCCs (cardiac)	Mouse	Ascending aorta	*Mef2c-Cre* transgene and *ROSA26-LacZ* reporter allele	Cre/lox recombination	Sawada et al. (2017)
	NCC (cranial)	Chick	Branchial arches	Fluorescence microscopy in fixed embryos	DiI labeling	Lumsden et al. (1991)
	NCCs (cranial and cardiac)	Chick and quail	Branchial arch arteries	Chicken (*Gallus gallus*)-Japanese quail chimeras	Isotopic and isochronic grafts	Le Lièvre and Le Douarin (1975)
	Pro-epicardium	Chick and quail	Coronary vasculature	Chicken (*Gallus domesticus*)-Japanese quail chimeras	Isotopic and isochronic grafts	Gittenberger-de Groot et al. (1998)
		Chick	Coronary vasculature	Targeting *in ovo* or tagging dissected pro-epicardial cells *in vitro* followed by transplantation	DiI labeling, retrovirus encoding β-galactosidase	Mikawa and Gourdie (1996)
		Mouse	Coronary vasculature	Gata5-Cre transgene and PDGFRβ-lox/lox	Cre/lox recombination	Mellgren et al. (2008)
				Gata5-Cre transgene, PDGFRβ-lox/lox; WT1-Cre, R26R-YFP; WT1-Cre and PDGFRα-GFP	Cre/lox recombination	Smith et al. (2011)
	Sclerotome	Chick and quail	Descending aorta	Chicken (*Gallus domesticus*)-Japanese quail chimeras	Homotopical and unilateral grafts	Wiegreffe et al. (2007)
		Chick and quail	Descending aorta	Chicken (*Gallus gallus*)-Japanese quail chimeras	Orthotopic, isochronic, unilateral or bilateral grafts	Pouget et al. (2006)
		Mouse	Descending aorta	*Meox1-Cre* transgenic and *ROSA26-LacZ* reporter allele	Cre/lox recombination	Wasteson et al. (2008)
		Zebrafish	vSMCs	*Tg(ola-twist1:gal4); Tg (UAS:Kaede)*	Gal4:UAS transgenesis and photoconversion	Stratman et al. (2017)
	Secondary heart field	Chick and quail	Base region of the aorta and the pulmonary trunk	Chicken (*Gallus gallus*)-Japanese quail chimeras	Isotopic and isochronic grafts	Waldo et al. (2005)
		Human embryo	Base region of the aorta and the pulmonary trunk	None available	Immunohistochemistry	Yang et al. (2013)

Continued

Development (2021) 148, dev197384. doi:10.1242/dev.197384

Table 2. Continued

Tissue	SMC progenitor source	Vertebrate model	Target tissues	Tools	Techniques	Reference
		Mouse	Ascending aorta	*Wnt1-Cre* transgene and ROSA26-LacZ reporter allele	Cre/lox recombination	Sawada et al. (2017)
Respiratory system	Pulmonary mesenchymal cells	Mouse	Airway branches	*mWt1IRES/GFP-Cre* (*Wt1-Cre*) and *R26R-EYFP*	Cre/lox recombination	Cano et al. (2013)
				Wt1-Cre	Cre/lox recombination	Dixit et al. (2013)
				Tbx4^{LME}-Cre	Cre/lox recombination	Kumar et al. (2014)
Intestinal system	Lateral plate mesoderm	Zebrafish	Intestinal tube	Tg(hand2:EGFP)^{pd24}	Tol2-mediated transgenesis and confocal microscopy	Gays et al. (2017); Yin al. (2010)
				Tg(draculin:creERT2)	Cre/lox recombination	Prummel et al. (2020); Gays et al. (2017)
		Chick and quail	Midgut	Cultured chicken midgut explant	*Ex vivo* organ manipulation	Huycke et al. (2019)

ENU, N-ethyl-N-nitrosourea; NCC, neural crest cell; PC, pericytes; vSMC, vascular smooth muscle cell.

dorsal region of the DA, and begin also to express transgelin. This population, referred to as 'secondary SMCs', migrates along the ventral region of the DA, replacing the primary SMCs located in the ventral region of the vessel. The reason for two different populations is unknown; however, it has been suggested that a dual origin for cells that support the aortic wall could be advantageous during animal evolution, because failure of tissue to develop from one origin could be compensated for by the second origin (Wiegreffe et al., 2009).

Although NCCs give rise to vSMC progenitors at the level of the ascending aorta, SMCs in the heart vasculature have different developmental origins. Using quail-to-chick chimeras and human embryos, it has been demonstrated that, after the formation of the septation of the aortic sac, cells derived from the secondary heart field (SHF) surround the base region of the aorta and the pulmonary trunk and begin to express ACTA2 (Waldo et al., 2005; Yang et al., 2013) (Fig. 1). Using *ROSA26:LacZ* reporter mouse lines crossed with *Wnt1-Cre* and *Mef2c-Cre* animals (for cardiac NCC and SHF tracing, respectively), the SHF has been shown to contribute to the outer medial cells of the ascending aorta, while the inner medial cells are derived from cardiac NCCs (Sawada et al., 2017).

A further source of vSMC for the coronary vasculature in mammals is the pro-epicardium, a transient embryonic structure located dorsally in the developing heart tube. After the formation of the epicardial layer in the surface of the developing heart, epicardial cells undergo epithelial-to-mesenchymal transition (EMT), migrate into the heart and provide vSMC progenitors (Gittenberger-de Groot et al., 1998; Mellgren et al., 2008; Mikawa and Gourdie, 1996; Smith et al., 2011; Vrancken Peeters et al., 1999) (Fig. 1).

In addition to chick and mouse models, the zebrafish has become another important system with which to study the origin and function of vascular mural cells during cardiovascular development (Santoro et al., 2009). ACTA2 and transgelin expression can be seen as early as 60 hpf (hours post-fertilization) around the trunk vasculature and transgenic zebrafish reporter lines can mark vSMC throughout adulthood (Santoro et al., 2009; Whitesell et al., 2014). Using mural cell-specific elements [such as the *acta2* promoter/enhancer element, *sm22α-b* (*transgelin1*) enhancer element, the *abcc9* promoter and the *pdgfrb* promoter], different groups have been able to trace vSMC origin and differentiation in living zebrafish (Ando et al., 2016, 2019; Chen et al., 2017b; Whitesell et al., 2019, 2014). Similar to mammals, zebrafish vSMC derive from the lateral plate mesoderm and, more precisely, from a population of cells adjacent to the sclerotome region (Kelley et al., 2019; Santoro et al., 2009; Stratman et al., 2017). The advantages of the zebrafish system for live imaging have also shown the contribution of hemodynamics and NOTCH signaling for the recruitment of vSMC to the dorsal aorta (Ando et al., 2019; Chen et al., 2017b).

A final source of vSMCs is the mesenchyme, which also contributes to airway and intestinal SM lineages (discussed below). Using *Cre* reporter mouse lines, it has been shown that WT1^{+} and Tbx4^{+} mesenchymal lineages contribute to distinct subpopulations of bronchial SMCs and vSMCs, which are dependent on active Hh signaling (Dixit et al., 2013; Zhang et al., 2013; Moiseenko et al., 2017). Parallel studies have demonstrated that WT1^{+} serosal mesothelial cells can also give rise to SM of all major blood vessels in the mesenteries and gut (Wilm et al., 2005).

Pericytes

Although pericytes share similarities with vSMCs in terms of gene expression markers, functionally they are very different. The role of pericytes is to control the formation and permeability of the blood-brain barrier (BBB) (Armulik et al., 2010; Daneman et al., 2010), regulate capillary blood flow (Hall et al., 2014) and, finally, control vessel stabilization (Lindahl et al., 1997a).

While vSMCs wrap around large endothelial tubes, producing basement membranes located between the SMC and EC, pericytes are solitary cells located sporadically along the arterioles, venules and capillaries (Armulik et al., 2011). They do not completely cover the smaller vessels and instead extend primary and secondary cytoplasmic processes along the surface of the endothelium (Hartmann et al., 2015) (Fig. 1). Furthermore, pericytes are embedded within the vascular basement membrane with a peculiar rounded cell body, long dendrite-like process and attached to the longitudinal axis of capillaries (Sims, 1986; Attwell et al., 2016). Pericytes interact with ECs both physically, by means of gap junctions, and through paracrine signaling (Armulik et al., 2005; Gaengel et al., 2009).

Although pericytes and vSMCs share some markers, specific markers can be used to distinguish between them (Table 1).

Development (2021) 148, dev197384. doi:10.1242/dev.197384

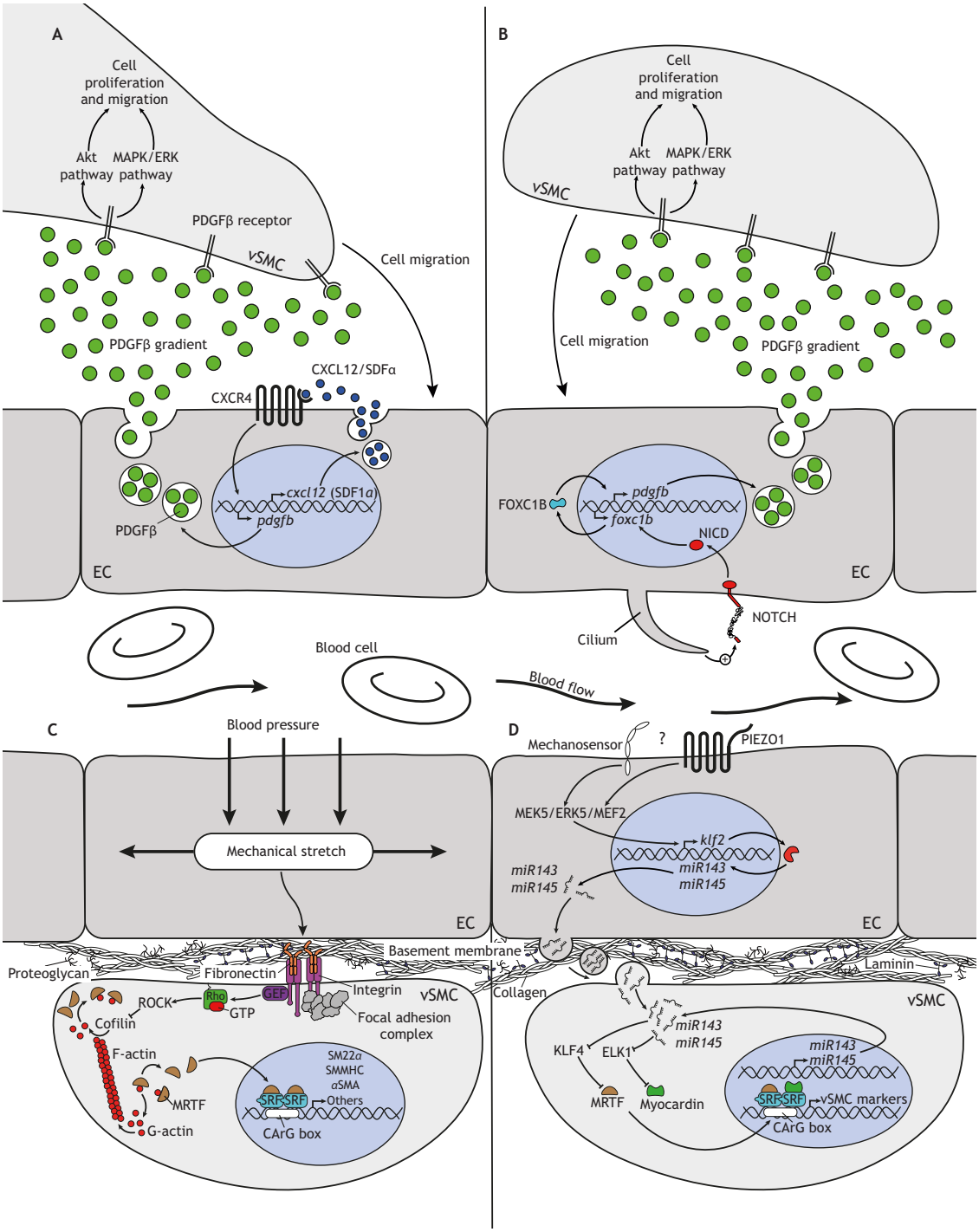

Fig. 2. Signaling and mechanical forces involved in vSMC recruitment and differentiation. Schematic regulation of cell signaling pathways involved in the recruitment and differentiation of vascular smooth muscle cells (vSMCs). (A) In zebrafish endothelial cells (ECs), an autocrine CXCR4/CRCL12 chemokine signaling axis promotes vSMC recruitment through an increase of PDGFβ secretion. The PDGFβ gradient produced by ECs stimulates vSMCs to migrate in the direction of this cue. (B) Cilia on zebrafish ECs sense blood flow and lead to activation of the NOTCH pathway, which induces the transcription factor FOXC1B to promote vSMC recruitment, possibly through secretion of PDGFβ molecules. (C) It has been shown that stretching the vascular wall may induce vSMC differentiation while they are in contact with ECs; indeed, mechano-stretch by blood flow stimulates the Rho/ROCK pathway through integrin and fibronectin interaction, and promotes actin polymerization by inhibiting cofilin. In this way, myocardin-related transcription factors (MRTFs) are released by globular actin (G-actin) and translocate into the nucleus. Interaction of MRTF with serum response factor (SRF) promotes transcription of different SMC markers, such as SM22α, SMMHC and α-SMA. (D) Blood flow induces transcription of KLF2 through the MEK/ERK/MEF pathway in ECs. The mechanosensor involved in this process is unknown, but PIEZO1 is a possible candidate. In turn, KLF2 induces transcription of *miR143* and *miR145*, which are transported through extracellular vesicles secreted by ECs in vSMCs where they induce inhibition of KLF4 and ELK1. In this way, myocardin and MRFT can translocate into the nucleus and, in association with SRF, induce the transcription of vSMC markers. αSMA, α smooth muscle actin; CXCR, chemokine receptor; CXCL, chemokine ligand; ERK, extracellular signal-regulated kinase; EIK1, ELK, ETS domain-containing protein Elk1; FOXC1B, forkhead box C1B; KLF, Kruppel-like factor; MAPK, mitogen-activated protein kinase; MEF, myocyte enhancer factor; NICD, NOTCH intracellular domain; PDGF, platelet-derived growth factor; SM22α, transgelin; SMMHC, smooth muscle-myosin heavy chain;

Development (2021) 148, dev197384. doi:10.1242/dev.197384

In mammals, these markers include platelet-derived growth factor receptor β (PDGFRβ), which is involved in the proliferation and recruitment of pericytes (Hellstrom et al., 1999; Olson and Soriano, 2011), the nerve-glial antigen 2 (NG2), which is involved in the recruitment of pericytes to the BBB and tumor vasculature (Ozerdem et al., 2001; Stallcup, 2018), the GTPase activating protein regulator of G-protein signaling 5 (RGS5) (Mitchell et al., 2008), and CD146, a transmembrane glycoprotein that functions as an adhesion molecule (Chen et al., 2017a).

Although pericytes play a crucial role in the vascular development, maturation, stabilization and remodeling, their developmental origin is heterogenous. Pericytes in the mouse liver (Asahina et al., 2011), lung (Que et al., 2008) and gut (Wilm et al., 2005) have been traced from the mesothelium, an epithelial monolayer that lines the lung. Pericytes in most other organs originate from the ectoderm. Chick-quail chimera analyses indicate that pericytes in the central nervous system originate from NCCs (Etchevers et al., 2001). Supporting this, genetic lineage-tracing experiments in zebrafish and mice using neural crest-specific *Cre* recombinase transgenic lines (such as *Wnt-1-Cre* and *Sox10-Cre* in combination with *loxP*-mediated fluorescent reporter line) have demonstrated that brain pericytes are neural crest derived (Ando et al., 2016; Foster et al., 2008). Trans-differentiation from ECs into pericytes has also been suggested to occur in birds (DeRuiter et al., 1997), although this is not a major route of pericyte formation during embryonic development in other vertebrates (Santoro et al., 2009). Recent work in the zebrafish has confirmed that the pericytes that interact with vessels in the brain are derived from NCCs, except for those in the hindbrain, where pericytes are instead derived from the mesoderm (Ando et al., 2016) (Fig. 1). Interestingly, genetic fate-mapping analysis in embryonic mice has revealed that tissue myeloid progenitors give rise to pericytes in the skin, by showing that the number of pericytes is reduced in *Pu.1* (*Spi1*$^{-/-}$) homozygous knockout mice, which lack myeloid progenitors (Yamazaki et al., 2017).

Overall, the ontogeny of pericytes and vSMCs is very distinct from that of other specialized SMC types and recent genome-wide quantitative transcriptomes studies have highlighted the complexity of this mural cell population in adult organs (Muhl et al., 2020; Vanlandewijck et al., 2018). Interestingly, single-cell transcriptomic analyses of the adult mouse brain have led to the identification of a population of perivascular fibroblast-like cells that are present on all vessel types, except capillaries (Vanlandewijck et al., 2018).

Smooth muscle cells in the respiratory system

SMCs that surround the respiratory system are called airway smooth muscle cells (aSMCs). aSMCs encircle the bronchial tree, and play a vital role in airway structure and function, such as in the regulation of bronchomotor tone and in the control of the airway caliber (Stephens, 2001). They form layers that circumferentially surround the bronchial tree, with differences in organization between the upper and lower airways (Amrani and Panettieri, 2003) (Fig. 1). Moreover, aSMCs contribute to the support and homeostasis of the lung function during embryonic development (Jesudason, 2009). aSMC peristalsis (involuntary wave-like contractions) has been observed in a number of species ranging from chicks to humans (McCray, 1993; Parvez et al., 2006), and early impairment in differentiation of this cell lineage can lead to respiratory dysfunction at birth (Lindahl et al., 1997b). aSMC express similar markers to vSMCs, such as ACTA2, SM-MHC, desmin, SRF and myocardin (Table 1).

Several studies have reported that aSMCs are derived from mesenchymal progenitors that relocate around the developing lung epithelium (El Agha et al., 2014; Yang et al., 1999). Early reports have suggested that during mammalian embryogenesis, aSMCs originate from NCCs and mesenchymal cells before vSMCs (Low and White, 1998). Furthermore, studies in mice using a *LacZ-Fgf10* transgenic reporter line, which specifically labels the distal lung mesenchyme, have revealed that *Fgf10*$^{+}$ cells include progenitors of the parabronchial SMCs (Mailleux et al., 2005). Another study has indicated that a subset of aSMCs cells might originate from proximal lung mesenchyme (Shan et al., 2008). By using the *Wt1-Cre*^{ERT2} transgenic line to lineage trace and visualize mesothelial cells during murine development and differentiation, Dixit and colleagues have been able to show that *Wt1*$^{+}$ mesothelial cells migrate and enter the fetal lung to provide different lung progenitors, such as aSMCs, vSMCs and fibroblasts (Cano et al., 2013; Dixit et al., 2013). More recently, however, an *in vivo* lineage-tracing study using reporter lines that mark different progenitors has shown that *Fgf10*$^{+}$ (distal lung mesenchyme) and *Wt1*$^{+}$ (mesothelium) provide only a minor contribution to the SMC lineage, whereas *Axin2*$^{+}$ and *Gli1*$^{+}$ cells, which are enriched in sub-epithelial mesenchyme, produce most of the aSMCs during embryonic development, but with low specificity (Moiseenko et al., 2017).

Organ culture and single-cell studies have described that such mesenchymal progenitor niches are in close proximity to the budding and bifurcating airway branches (Kim et al., 2015; Kumar et al., 2014). Furthermore, recent studies coupling genetic lineage tracing, single-cell RNA sequencing and organoid culture have indicated that, in the lung, a subset of mesenchymal cells express LGR5 and LGR6, known markers of epithelial stem cells. A specific subset of these mesenchymal cells (LGR6^{+}) are progenitors for aSMCs and promote airway differentiation of epithelial progenitors via WNT-FGF10 cross talk (Lee et al., 2017). During development, the lung mesoderm also generates vSMCs. Recent findings have revealed that EZH2 is a crucial molecular determinant required to restrict SM differentiation in the developing lung mesothelium (Snitow et al., 2016).

Overall, compared with vSMCs, the embryonic origins of aSMCs have been less intensively studied. Considering the different experimental approaches and transgenic lines that have been used, the extent to which mesenchymal and mesothelial progenitors contribute to the aSMC lineage is far from understood. Further studies are still needed to unveil and elucidate the complete process of airway myogenesis, as well as to decode how the integration of mechanical and biological signaling shape this tissue.

Smooth muscle cells in the intestinal system

SMCs embedding the walls of visceral organs are commonly called intestinal (or visceral) smooth muscle cells (iSMCs). They regulate peristalsis: the involuntary movement of the longitudinal and circular muscles that occur in progressive wavelike contractions in esophagus, stomach and intestines allowing movement of food through the digestive system. Studies in chicken and zebrafish embryos have described the emergence, propagation and physiological-molecular development of SM-dependent gut peristalsis in the lower digestive tract (Abrams et al., 2012; Chevalier et al., 2020, 2017; Gays et al., 2017). During murine embryonic development, it has been shown that iSMCs confer shape and mobility of the intestine through peristaltic movements (Gabella, 2002), facilitated by the presence of circumferential and

Development (2021) 148, dev197384. doi:10.1242/dev.197384

longitudinal layers of iSMCs that wrap around the gut tube (Kedinger et al., 1990). The events leading to this final anatomical structure of SM around the gut have been clearly described in mammals: an inner layer of circumferentially smooth muscle is established and, later, an outer longitudinal layer is formed and aligned (McHugh, 1995, 1996).

In vertebrates, endodermal epithelium is surrounded by layers of SMCs derived from splanchnic mesoderm. After the closure and maturation of the epithelium, the splanchnic mesoderm gives rise to mesenchymal cells that surround the primitive gut and form two different layers of SM with the circumferential and longitudinal orientations. In the mouse, circular iSMCs differentiate at E13, while longitudinal layers differentiate at E16 (Walton et al., 2016). In addition, in chicken, circular and longitudinal iSMCs do not differentiate simultaneously, with circular iSMCs developing at E6 and longitudinal iSMCs at E13 (Le Guen et al., 2015). Recent studies have helped to understand how the muscle layers are specified at the correct time and with the correct orientation (Huycke et al., 2019), and the development of iSMCs in the human gastrointestinal tract (Wallace and Burns, 2005).

The development and patterning of iSMCs have also been studied and characterized in the zebrafish model, which has many features that can be considered homologous to amniotes. The zebrafish intestine has a single layer of epithelial cells facing the lumen attached to mesenchyme and surrounded by circular and longitudinal SM. Here, circular layers of iSMCs develop and differentiate at around 72 hpf, while longitudinal layers develop at 120 hpf (Georgijevic et al., 2007; Wallace et al., 2005). Recently, genetic lineage tracing of zebrafish embryos has shown that visceral iSMCs originate from the lateral plate mesoderm (Prummel et al., 2020). Studies in zebrafish embryos have also shed light on early embryonic events leading to the formation of the first circular layer of iSMCs (Horne-Badovinac et al., 2003). It has been shown that the lateral plate mesoderm undergoes a partial EMT; unlike canonical EMT, lateral plate mesoderm migrates as a cohesive layer of mesenchymal cells, retaining features of both epithelial (such as cell-cell adhesions) and mesenchymal (such as the ability to migrate) cells, surrounding first the dorsal and then the ventral region of the primitive gut (Gays et al., 2017). Although the precise signals driving such cellular interactions remain to be determined, a variety of extracellular matrix molecules (e.g. laminin) and growth factors (e.g. transforming growth factor β TGFβ), have been shown to be involved during this process during zebrafish embryonic development (Gays et al., 2017; Yin et al., 2010).

Signaling and mechanical forces involved in smooth muscle cell differentiation

Once SMC progenitors are established, they then differentiate and cover the tissue of interest in a process called myogenesis. Myogenesis not only involves canonical cell signaling, but also mechanical forces that drive SMC differentiation and recruitment (Table 3).

Vascular mural cells

Studies in vertebrates have shown that different signaling pathways, such as PDGF, TGFβ, NOTCH, Sonic hedgehog (Shh), WNT and chemokine/NF-kB signaling, are involved in mural cell (vSMC and pericyte) differentiation, recruitment and stabilization. As most of these pathways have been reviewed in detail previously (Armulik et al., 2011; Badimon and Borrell-Pages, 2017; Döring et al., 2014; Gaengel et al., 2009; Goumans and Ten Dijke, 2018), we focus on the most exciting ones.

Disruption of PDGFRβ signaling strongly affects mural cells recruitment and causes abnormal aortic expansion and elasticity in vertebrates (Hellstrom et al., 1999; Olson and Soriano, 2011; Shimada et al., 1998; Stratman et al., 2017). An interesting signaling crosstalk between PDGF, the CXCR4 chemokine receptor and its associated ligand CXCL12 (also known as SDF1α) has been proposed, because SDF1α expression has been shown to be stimulated by PDGFβ (Song et al., 2009). Knockout of *Cxcr4* in mice results in defective arterial patterning with associated defects in mural cell coverage of the vasculature (Li et al., 2013). Additional evidence for an involvement of the SDF1α/CXCR4 axis in vSMC recruitment has recently been provided in zebrafish (Fig. 2). Here, it has been shown that *sdf1a*/*cxcr4* zebrafish mutants have fewer vSMCs associated with the dorsal aorta, suggesting that SDF1α/CXCR4 autocrine activity leads to increased production of PDGF by ECs (Stratman et al., 2020). Overall, chemokine signaling in vSMC recruitment and vascular maturation is evolutionarily conserved (Stratman et al., 2020; Li et al., 2013; Song et al., 2009).

Hemodynamic forces (also called shear- or mechano-stress) are essential for the vascular maturation and the induction of vascular myogenesis in vertebrates (Chen et al., 2017b; Wu et al., 2008). The gene encoding the transcription factor KLF2 is one of the best known flow-response genes (Huddleson et al., 2004). Studies using *Klf2*-null mice indicate that KLF2 is required for vSMC migration, and have elucidated a novel mechanism involving communication between PDGF and CXCR4 in vascular maturation (Wu et al., 2008). Studies in zebrafish further support the role of shear stress on vascular myogenesis (Chen et al., 2017b). After arterialization, blood flow causes hemodynamic forces that stimulate endothelial primary cilia to activate Notch signaling, as shown by previous studies that have reported the ciliary localization of *Notch1* and *Notch3* receptors (Ezratty et al., 2011; Leitch et al., 2014). Endothelial Notch activation leads then to *Foxc1b* expression, which promotes vSMC recruitment and differentiation (Chen et al., 2017b). Our knowledge of shear stress in driving mural cell recruitment is limited and requires further characterization.

Interestingly, expression of two master regulators of mural cell differentiation, SRF and microRNAs (miRNAs or miRs), are also mechanosensitive (Kumar et al., 2014; Turczyńska et al., 2013). For example, *ex vivo* experiments in mouse portal veins have shown a role for stretching of the vascular wall in driving the expression of vSMC markers, mediated by Rho activity and actin polymerization through a SRF-dependent mechanism (Albinsson et al., 2004) (Fig. 2). Indeed, during vSMC differentiation, many signaling pathways converge on the regulation of SRF and its co-factor myocardin and myocardin-related transcription factors (MRTFs) (Olson and Nordheim, 2010; Owens et al., 2004). SRF and myocardin form a key regulatory complex for vSMC differentiation by binding to CArG box DNA elements, thereby regulating vSMC-specific markers (Majesky, 2007) (Fig. 2). A lack of, or reduction in, SRF has been directly linked to a SMC phenotype characterized by impairment of SM differentiation, proliferation and migration in both the cardiovascular and gastrointestinal systems (Browning et al., 1998). Similarly, myocardin loss of function is lethal in mouse embryos due to the lack of vSMCs in the dorsal aorta, which leads to severe vascular abnormalities (Amrani and Panettieri, 2003). Seminal work has suggested that actin polymerization triggers the nuclear localization of MRTF to stimulate SRF-dependent transcription of the master regulators involved in SMC differentiation (Miralles et al., 2003).

Development (2021) 148, dev197384. doi:10.1242/dev.197384

Table 3. Signaling pathways and molecules involved in smooth muscle cell development and differentiation

Tissue	Signaling pathway	Molecule	SMC function	Reference
Vascular	PDGF	PDGFR	Pericyte recruitment	Olson and Soriano (2011)
		PDGF-BB	SMC recruitment	Hellstrom et al. (1999); Stratman et al. (2017)
	TGFB	TGFβ	SMC differentiation	Grainger et al. (1998); Chen and Lechleider (2004)
	NOTCH	NOTCH2/3	SMC differentiation	Wang et al. (2012)
		NOTCH3	Expansion of the brain pericytes	Wang et al. (2014)
		NOTCH	vSMC recruitment and differentiation	Chen et al. (2017a,b)
	EGF	HB-EGF	SMC recruitment	Iivanainen et al. (2003)
			Pericyte recruitment	Stratman et al. (2010)
	SHH	SHH	SMC progenitor recruitment	Dixit et al. (2013)
			SMC differentiation	Miller et al. (2004)
	WNT	WNT7B	Mesenchymal differentiation in SMC lineage	Cohen et al. (2009)
			SMC development and homeostasis	Wang et al. (2005)
Lung	SHH	SHH	SMC differentiation	Miller et al. (2004)
			Differentiation of lung mesenchyme into smooth muscle	Weaver et al. (2003)
			Smooth muscle formation	Pepicelli et al. (1998)
			Smooth muscle specification	Wan et al. (2005)
			SMC progenitor recruitment	Dixit et al. (2013)
	BMP	BMP4	Parabronchial smooth muscle lineage induction	Mailleux et al. (2005)
			SMC differentiation	Weaver et al. (2003)
	WNT	WNT1	Migration, recruiment and smooth muscle cell differentiation	Kumar et al. (2014)
		WNT7B	Mesenchymal differentiation in SMC lineage	Cohen et al. (2009)
			SMC development and homeostasis	Wang et al. (2005)
	FGF	FGF10	Parabronchial smooth muscle lineage induction	Mailleux et al. (2005)
			Progenitor cell proliferation	Ramasamy et al. (2007)
		FGF9	Undifferentiated or multipotent state maintenance	Weaver et al. (2003)
			Prevent differentiation of the mesenchymal cells into smooth muscle cells	del Moral et al. (2006)
	PDGF	TNC	Proliferation of pulmonary SMC precursors and differentiation	Cohen et al. (2009)
Intestine	TGFβ	ALK5	Migration of SMC progenitors	Gays et al. (2017)
	SHH	SHH	SMC lineage specification	Cotton et al. (2017)
			Delineation of concentric and orthogonal layers of SMCs	Huycke et al. (2019)
			Growth of mesenchymal progenitors	Mao et al. (2010)
		SHH/IHH	SMC development	Ramalho-Santos et al. (2000)
	Hippo	YAP/TAZ	Spatial-temporal SMC differentiation	Cotton et al. (2017)
	MYOCD	MYOCD	SMC lineage specification	Cotton et al. (2017)
	BMP	BMP2, BMP7, NOGGIN and GREM1	Delineation of concentric and orthogonal layers of SMCs	Huycke et al. (2019)

ALK5, TGFβ type I receptor; BMP, bone morphogenetic protein; EGF, epidermal growth factor; FGF, fibroblast growth factor; HB-EGF, heparin-binding EGF-like growth factor; IHH, Indian hedgehog; PDGF, platelet-derived growth factor; PDGFR, PDGF receptor; SHH, sonic hedgehog; SMC, smooth muscle cell; TGFβ, transforming growth factor β; TNC, troponin C; YAP, yes-associated protein 1.

Similarly, numerous studies have identified that miRs are potent regulators of vSMC biology. Among these, miR-143 and miR-145 control vSMC differentiation. Indeed, Boettger and colleagues have reported that the *mir-143/145* gene cluster promotes a contractile phenotype of murine vSMCs (Boettger et al., 2009). Furthermore, it has been shown by *in vivo* and *in vitro* studies that miR-145 and miR-143 regulate vSMC fate and plasticity by directly targeting a network of transcription factors, including KLF4 (Kruppel-like factor 4), myocardin and ELK1 (member of ETS oncogene family), and dysregulating the TGFβ signaling cascade, including SMAD2, SMAD3 and TGFβ ligands (Cordes et al., 2009; Zhao et al., 2015) (Fig. 2). A role for miR-143 and miR-145 in mediating the communication between vSMCs and ECs to modulate angiogenesis and vessel stabilization has also been proposed (Climent et al., 2015). Moreover, it has been shown that miR-143 and miR-145 are among those mechano-sensitive miRNAs, and the *mir-143/145* cluster is regulated through KLF2 (Hergenreider et al., 2012). Here, the authors have proposed a mechanism through which flow promotes the transfer of endothelial-derived miR-143/145 to adjacent mural cells via extracellular vesicles. Another miRNA, miR26a, has been proposed to regulate mural cell differentiation and function, both in zebrafish and in mice (Leeper et al., 2011). Although the mechanism and the cell-autonomous function of this miRNA is still controversial, miR-26a acts at the intersection between PDGF and the BMP/TGFβ family during vSMC maturation (Watterston et al., 2019; Yang et al., 2017).

The molecular mechanisms that regulate epigenetic control of vSMC differentiation are also currently under intense study, but many pathways and factors still need to be identified. Innovative new tools and approaches, such as single-cell RNA sequencing and epigenetic assays, are allowing the identification of mural cell heterogeneity (Vanlandewijck et al., 2018), specific intermediate

Development (2021) 148, dev197384. doi:10.1242/dev.197384

(progenitor) cell subpopulations of mural cells (Liu et al., 2019), as well as novel molecular determinants of vSMC during development and disease (Muhl et al., 2020; van Kuijk et al., 2019).

Airway smooth muscle cells

For aSMCs, the mesenchymal progenitor niches are located at the branch tip of the developing lung, and different studies have demonstrated a role for epithelial-derived morphogens in their recruitment and differentiation (Kumar et al., 2014; Mailleux et al., 2005). Although the precise mechanisms are not yet completely defined, different studies have demonstrated the involvement of FGF9, FGF10, SHH, BMP and WNT signaling in this process (Fig. 3). Expression of these molecules at the level of the pulmonary mesothelium, airway stalks and mesenchymal progenitors leads to aSMC recruitment, differentiation and envelopment of the epithelium. Blocking FGF9 signaling by knocking out its receptors in the airway mesenchyme results in ectopic SM differentiation and impaired epithelial morphogenesis (Yi et al., 2009). Mutant mice lacking *Shh*, which is normally expressed in the embryonic lung epithelium at the distal bud tips, show an absence of aSMCs (Miller et al., 2004), while exogenous recombinant *Shh* induces aSMC differentiation (Weaver et al., 2003). Fgf10 hypomorphic embryos have diminished aSMCs recruitment, although this may be an indirect effect due to impaired Shh epithelial expression (Ramasamy et al., 2007). FGF10 is expressed by early SMC progenitors and is involved in BMP4 expression at the level of the epithelium, which is required for entry into the aSMC lineage (Mailleux et al., 2005). The interplay between FGF10 and SHH signaling during mesenchymal differentiation and lung morphogenesis has been investigated, but remains unclear (Herriges et al., 2015) (Fig. 3).

Although these signaling cues are necessary for SMC differentiation, they are not sufficient. Yang and colleagues have demonstrated that, in absence of mechanical tension, mesenchymal progenitors do not differentiate in the SM lineage, while mechanical stretch (in the form of cell spreading, elongation or stretching) induces the expression of SM markers (Yang et al., 2000); αSMA, SM22α and desmin expression levels increase when uniaxial continuous stretching is induced through the use of a silastic membrane, and in *ex vivo* experiments, the numbers of αSMA-positive cells surrounding the bronchial tree increases. The authors have identified that SRF and its antagonist SRFΔ5 are key players involved during this tension-induced myogenesis, which has been confirmed by analyzing two different human hypoplastic lung pathologies (Yang et al., 2000). The same group have also identified a set of stretch-responsive factors, designated as tension-induced/inhibited proteins (TIPs), that are able to promote myogenesis possibly through chromatin remodeling and histone acetylation (Jakkaraju et al., 2005).

Intestinal smooth muscle cells

Numerous signals are implicated in differentiation of SMCs in developing gut (Fig. 4). In particular, endodermal-derived SHH and Indian hedgehog (IHH) signaling act on the surrounding mesenchyme to control its differentiation into iSMCs (Kim and Choi, 2009; Mao et al., 2010). However, opposing results have been obtained from chicken versus mouse. Tissue-grafting experiments and pharmacological modulation of the Hedgehog (Hh) pathway in chick explant culture suggests that this pathway inhibits SM differentiation (Sukegawa et al., 2000). Conversely, studies in mouse reveal that Hh signaling promotes SM formation via the direct activation of myocardin, a master regulator of SM differentiation (Ramalho-Santos et al., 2000; Zacharias et al., 2011). Another secreted factor that is implicated in gut SM formation is BMP signaling (de Santa Barbara et al., 2005). It has been proposed that BMP4 inhibits gut myogenesis: BMP4 overexpression results in reduction of mesenchymal layers and decreased SM in developing chick gut. BMP4 is activated by and dependent on Hh secretion by the adjacent endoderm, suggesting a level of integration between the two pathways in this context (Roberts et al., 1998).

Recently, Huycke and colleagues have demonstrated that the correct orientation of the circumferential and longitudinal layers is driven by unique mechanical forces and Shh signaling at different stages of gut development in chick. The authors have demonstrated that the orientation of the first (circumferential) SM layer is driven by residual strain created by differential growth of the mesenchyme, with cells in the inner mesenchyme having shorter cell cycles than cells of the outer mesenchyme (Huycke et al., 2019). Conversely, the longitudinal orientation is driven by cyclic contraction of the inner layer that induces SMCs of the outer layer to align perpendicular to the axis of strain. During this process, cell signaling involving SHH, BMP and BMP antagonists (such as noggin and gremlin 1) orchestrate the timing and location of these SM layers along the gastrointestinal tract (Huycke et al., 2019) (Fig. 4).

Studies in zebrafish have identified a crucial role for miR-145 in iSMC differentiation (Zeng et al., 2009; Zeng and Childs, 2012). miR-145 governs expression of BMP4 morphogens that drive both epithelial and SMC differentiation in intestine through autocrine and paracrine mechanisms (Zeng and Childs, 2012) (Fig. 4). TGFβ has been identified as a crucial player in the migration of SMC progenitors from the lateral plate mesoderm in the direction of developing tube (Gays et al., 2017). TGFβ acts through two distinct genes, *Zeb1* and *Foxo1*, that encode a zinc-finger and homeodomain transcription factor, and a Forkhead family transcription factor, respectively. During iSMC development and differentiation, ZEB1 (which is known to function in EMT) controls lateral plate mesoderm migration, while FOXO1A is involved in maintaining mesodermal progenitor stemness. TGFβ-induced miR-145 negatively regulates *zeb1* and *foxo1a* expression and, therefore, blocks EMT and mesodermal stemness; these biological processes need to be terminated in order to allow LPM differentiate in mature iSMCs (Gays et al., 2017) (Fig. 4).

The cardiovascular, respiratory and gastrointestinal systems are constantly subjected to mechanical stimuli; blood, gas and food create two types of mechanical forces on the internal wall of these organs: shear stress and pressure. Both forces affect the endothelium and epithelium, but also SMCs. A process called mechano-transcription is currently the focus of intense study. For example, it has been shown that vSMCs possess a mechanosensitive cell cycle regulation through the transcriptional regulation of p27Kip1 (Sedding et al., 2003), emerin and lamin A/C, two important components of nuclear envelope proteins localized beneath the inner nuclear membrane (Qi et al., 2016). It has been shown that mechanical forces are crucial also for iSMC differentiation by regulating transcription directly in these cells (Shi, 2017).

Some studies have shown a role for the mechano-sensors and mechano-transducers YAP and TAZ in growth and differentiation of gut mesenchyme (Cotton et al., 2017). The Hippo and YAP and TAZ pathways (YAP/TAZ) are regulated by mechanical cues and are involved in the regulating of the organ size and tumor growth (Dupont et al., 2011; Moya and Halder, 2019). Genetic analyses using mice genetic models have highlighted the functional interplay between Hippo and SHH pathways in gastrointestinal development (Cotton et al., 2017). Specifically, it has been proposed that

Development (2021) 148, dev197384. doi:10.1242/dev.197384

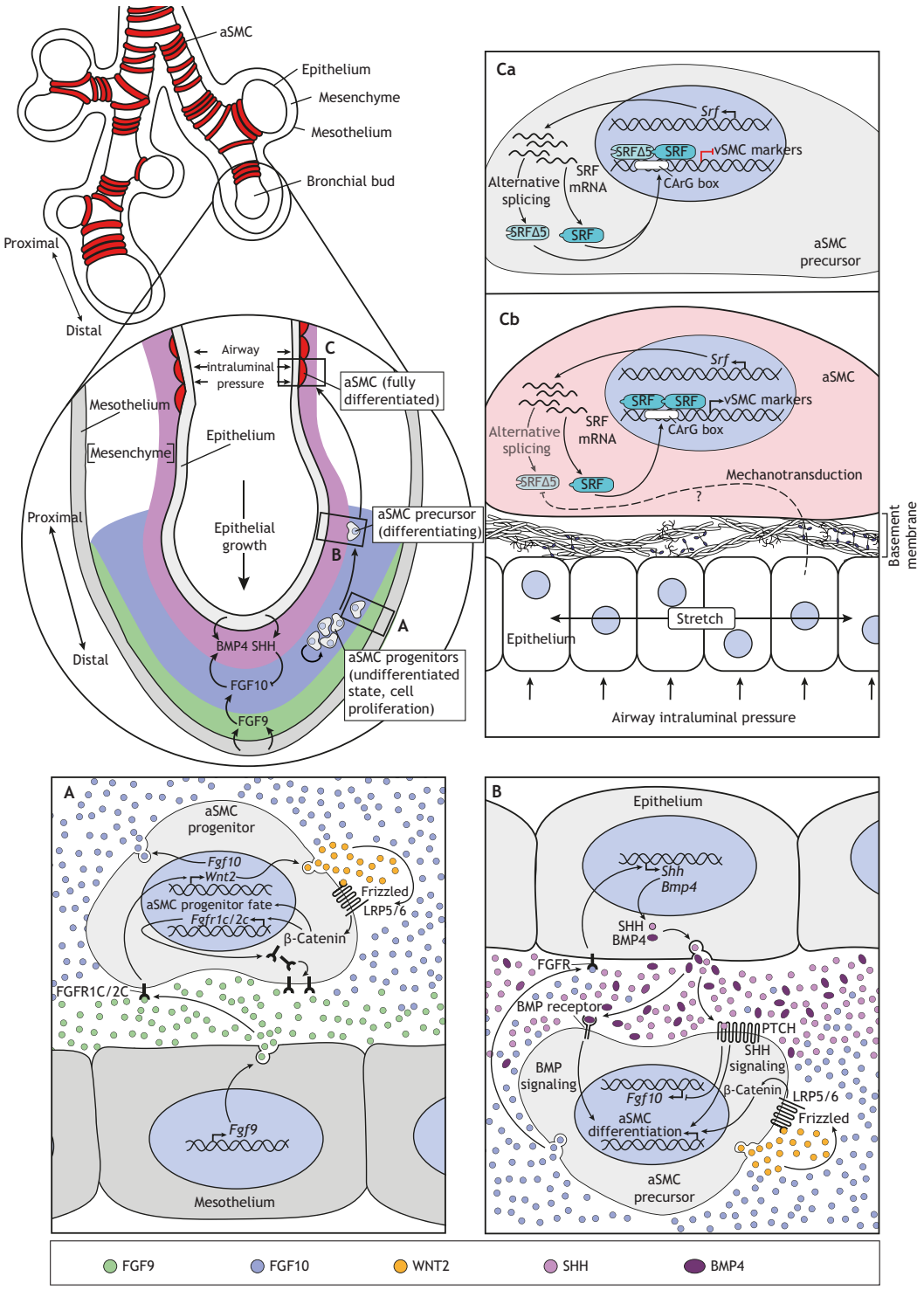

Fig. 3. Signaling driving aSMCs differentiation during lung development. During bronchial tree formation, the epithelium is embedded in mesenchyme and the mesothelium is the outermost layer. (A) At this stage, terminal buds are growing, driven by the expansion of the epithelial tube and mesenchyme. FGF9 secreted by mesothelium binds its receptor (FGF9R1c/2c) in submesothelial mesenchymal cells and induces the secretion of WNT2, which binds its receptors frizzled and LRP5/6. (B) WNT signaling induces the expression of FGF9R1C/2C in the mesenchymal cells in an autocrine manner and it reinforces responsiveness to FGF9. Furthermore, WNT signaling specifies submesothelium mesenchyme to adopt the airway smooth muscle cell (aSMC) progenitor fate. At the same time, FGF signaling drives the secretion of FGF10, which inhibits premature differentiation of the mesenchymal cells and induces their proliferation. FGF10 promotes the epithelial secretion of SHH and BMP4, which in turn inhibits FGF10 expression in mesenchymal cells. Furthermore, as the bronchial bud grows out, aSMC progenitors come into contact with SHH and BMP4, which drive progenitor differentiation. (Ca) In absence of stretch forces, alternative splicing induces the production of SRFΔ5 (a truncated form of SRF), which interacts with SRF in the nucleus and inhibits the transcription of SMC markers. (Cb) In the proximal region of the bronchial bud, aSMC precursors are in contact with the epithelium, and airway intraluminal pressure induces their stretch. This stretching inhibits the expression of SRFΔ5, and the SRF homodimer can induce SMC marker expression and drive their full differentiation. The molecular mechanism by which mechanotransduction drives repression of SRFΔ5 has not been fully elucidated. BMP, bone morphogenetic protein; FGF, fibroblast growth factor; FGFR, fibroblast growth factor receptor; SHH, sonic hedgehog; SRF, serum response factor.

Development (2021) 148, dev197384. doi:10.1242/dev.197384

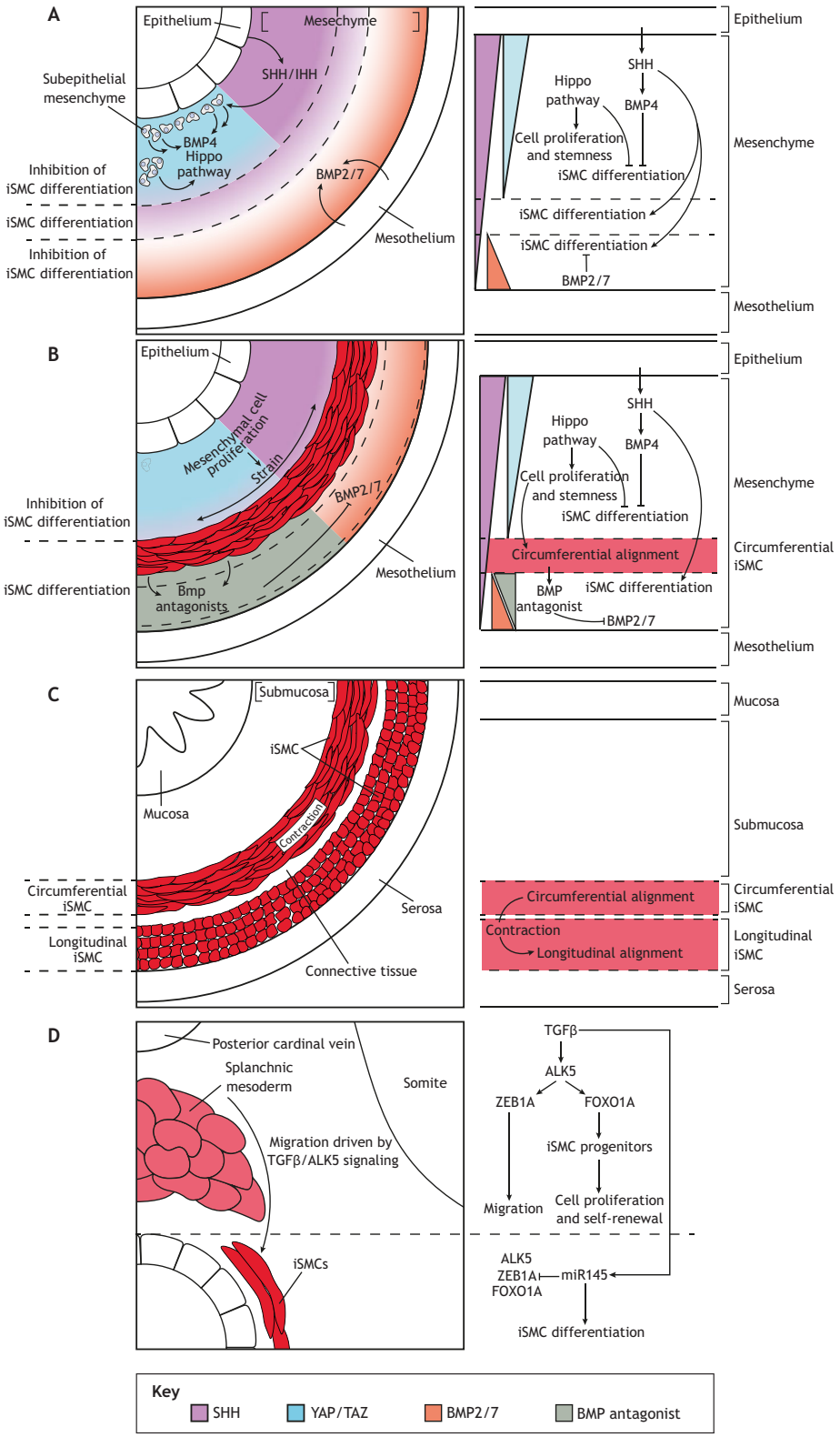

Fig. 4. Signaling pathways regulating iSMCs differentiation in gut development. During embryonic development, different signaling pathways coordinate the spatial-temporal control of intestinal (or visceral) smooth muscle cell (iSMC) differentiation. (A) During the early embryonic development, the intestinal tube is formed by an inner endodermal-derived epithelium, an outer mesothelial layer and the mesenchyme in the middle. SHH/IHH secreted by the epithelium induces the production of BMP4 in the subepithelial mesenchyme, while mesothelium induces secretion of BMP2/7. In coordination with the Hippo pathway, which promotes cell proliferation and stemness, these different gradients inhibit the differentiation of iSMC progenitors present in the mesenchyme, except in a middle region in which BMP concentration is low (region between dashed lines). Here, SHH/IHH signaling induces the differentiation of iSMC progenitors, which circumferentially align through mesenchymal cell proliferation that produces a strain induced by their amplification. (B) After the formation of the inner iSMC layer, it secretes BMP antagonists that inhibit BMP2/7 activity and induce the outer iSMC layer, which is driven by SHH/IHH signaling. (C) Spontaneous contractions of the inner smooth muscle layer induce outer iSMCs to longitudinally align. (D) TGFβ/ALK5 signaling induces expression of *zeb1a* and *foxo1a*. ZEB1A drives lateral plate mesoderm migration in the direction of the endodermal, while FOXO1A drives its proliferation and maintenance, preparing iSMC progenitors to their differentiation. Once lateral plate mesoderm migrates around endodermal-derived epithelium, TGFβ signaling induces transcription of *miR145*, which switches off *zeb1a* and *foxo1a* translation and induces iSMC differentiation. BMP, bone morphogenetic protein; IHH, Indian hedgehog; SHH, sonic hedgehog.

myocardin expression is driven by SHH signaling, but it is inhibited by YAP/TAZ, which spatially and temporally regulate iSMC differentiation (without affecting SHH transduction). When YAP/TAZ are downregulated in specific restricted zone, myocardin expression occurs, enabling iSMC lineage specification (Cotton et al., 2017) (Fig. 4).

Smooth muscle cells as drivers of organogenesis

Mechanical forces produced by the cellular environment have important roles in the differentiation and recruitment of SMCs. On the other hand, several studies have demonstrated that mechanical forces produced by SMCs instruct the morphogenesis of different organs during their development.

Development (2021) 148, dev197384. doi:10.1242/dev.197384

Although aSMCs are mainly known to play a crucial function postnatally, their role in lung morphogenesis has been studied in mammals (Kim et al., 2015). Indeed, localized SM differentiation contributes to shaping lung branches: the position and morphology of domain branches are highly stereotyped, as is the pattern of SM that differentiates around the base of each branch (Goodwin et al., 2019).

Shyer and colleagues have shown that differentiating SM layers play crucial roles in the formation of villi in the human and chick gut. The circular inner SM layer prevents expansion of the gut tube and causes compressive forces in the epithelium, which induce longitudinal ridges and intestinal folding. Meanwhile, the longitudinal outer SM layers fold the ridges into parallel zigzags, leading to villi formation (Shyer et al., 2013). These studies conclude that it is possible that the formation of human and chick villi occurs through similar steps. In addition to promoting villification, circular SM tone and contractions are also necessary for the anisotropic growth of the gut, which in turn is essential for high-aspect ratio of the organ, increased epithelial surface and compartmentalization of digestion (Khalipina et al., 2019).

Another important aspect to keep in mind is that the SMCs interact with their environment. Different *in vitro* studies have demonstrated that the co-culture of ECs and SMCs inhibits EC growth and movement by inducing EC quiescence. These responses are mediated by TGFβ action and abrogated VEGF responsiveness (Antonelli-Orlidge et al., 1989; Korff et al., 2001; Sato and Rifkin, 1989). Furthermore, Fortuna and colleagues have shown that, in zebrafish, vSMC recruitment to the dorsal aorta is required for the acquisition of noradrenergic specification by sympathetic precursors (Fortuna et al., 2015). Overall, SMCs support and regulate organ development and differentiation throughout the vertebrate body.

Perspectives and conclusions

In this Review, we have discussed how the SM of different organs share similarities, such as the expression of common markers and performing their function by constricting the hollow organs they ensheathe (e.g. circulatory, respiratory and digestive systems). However, specific differences do exist in the organization, origin, signaling between vSMC, aSMC and iSMC that reflect the specialization and functions of the organs and tissue with which they interact. The SMCs of different organs might be organized very differently and perform specialized functions, as well as expressing both specific and unique marker genes. Furthermore, the population of SMCs present within circulatory, respiratory and digestive systems are not homogenous; they have different origins and rely on different signals for specification. Overall, should we think of smooth muscle cells as a single cell type or do we need to consider them as heterogeneous populations? So far, we probably do not know enough about SMCs to fully address this question; we need to better understand their heterogeneity. It is these types of questions, however, that make SM a puzzling and exciting tissue to study.

The recent progress in understanding the origin, function and signaling of SMC development in a variety of organs can provide the basis for a better understanding, modeling and treatment of pathologies based on SMC abnormalities. For regenerative vascular medicine, there is a pressing need for new model systems to investigate vSMC development and heterogeneity (Sinha and Santoro, 2018). Induced-pluripotent stem cells (iPSCs) are an exciting, clinically relevant candidate for cell-based applied therapy. In particular, human IPSC-derived vSMCs are being used to regenerate or tissue-engineer both large vessels and the microvasculature (Cheung et al., 2012). Single-cell transcriptomics is emerging as an essential technique, enabling researchers to investigate different cell populations in more depth than ever before (Jakab and Augustin, 2020). As we have discussed, recent work has demonstrated that this new technology can be used to identify disease-relevant transcriptional signatures in vSMC-lineage cells in healthy blood vessels, with implications for disease susceptibility, diagnosis and prevention (Dobnikar et al., 2018).

In addition, aSMCs play an integral part in the pathogenesis of chronic airway diseases, such as asthma, where they contribute to airway remodeling and inflammation. Therefore, targeting the signaling pathways that regulate airway SM responses might provide new therapeutic approaches and treatments for chronic pulmonary disease.

The cellular plasticity of iSMCs is not only important for the normal differentiation and maturation of gastrointestinal SM, but also appears to play a significant role in a variety of intestinal diseases. Obstruction of the small intestine is a frequently encountered complication of many congenital and acquired gastrointestinal disorders (Gabella, 1990; Lin et al., 2012; MacDonald, 2008). Hypertrophy of the circular iSMCs in the constricted part of the intestine is the most common effect of the disease. Chronic partial obstruction of the small intestine can dramatically alter peristaltic contractile properties. We know relatively little about the molecular mechanisms that contribute to the phenotypic remodeling of intestinal SM in this obstructive disease (Chen et al., 2008; Shi, 2017). Mechanical stretch has been shown to alter gene transcription in iSMCs and this stretch-altered gene expression (e.g. mechano-transcription) could play a crucial role in pathogenesis of motility dysfunction and abdominal pain obstruction. It can be expected that future studies may be applicable to our understanding of the molecular events associated with motility dysfunction in chronic-intestinal pseudo-obstructions.

The anatomical organization and function of SMCs clearly demonstrates the complexity involved in understanding and decoding normal SM development in different organs. In recent years, important progress has been made in understanding the biology of SMCs, but many questions still remain unresolved: are there organ-specific and segment-specific patterns during smooth muscle development? How is the SMC molecular differentiation program integrated with the stemness program? Are there SMC stem/progenitor cells that reside in different organs, and can they be reactivated and eventually move through organs in embryos and adult? Does the SM of different organs develop in a manner similar to other SM tissues, i.e. vascular, respiratory and urogenital? How do the various SM layers of the mature vascular and gastrointestinal tract develop? Does organ metabolism influence their recruitment and differentiation? Such challenges need to be faced in the near future to support studies on SMC, as they represent a mysterious, but exciting, tissue in vertebrate bodies with great therapeutic potential.

Acknowledgements

We sincerely apologize to all our colleagues whose contributions to SMC biology we were unable to cite due to the complexity and extensiveness of the covered topics as well as space limitation. We thank the members of the Santoro lab for critical comments on the manuscript, as well as Ellen Jane Corcoran for editorial and language assistance.

Competing interests

The authors declare no competing or financial interests.

Funding

The authors' research was funded by a European Research Council Consolidator Grant (RENDOX-ERC-CoG 647057) as well as an Associazione Italiana Ricerca sul Cancro IG grant (20119).

References

Abrams, J., Davuluri, G., Seiler, C. and Pack, M. (2012). Smooth muscle caldesmon modulates peristalsis in the wild type and non-innervated zebrafish intestine. *Neurogastroenterol. Motil.* **24**, 288-299. doi:10.1111/j.1365-2982.2011.01844.x

Albinsson, S., Nordström, I. and Hellstrand, P. (2004). Stretch of the vascular wall induces smooth muscle differentiation by promoting actin polymerization. *J. Biol. Chem.* **279**, 34849-34855. doi:10.1074/jbc.M403370200

Amrani, Y. and Panettieri, R. A. (2003). Airway smooth muscle: contraction and beyond. *Int. J. Biochem. Cell Biol.* **35**, 272-276. doi:10.1016/S1357-2725(02)00259-5

Ando, K., Fukuhara, S., Izumi, N., Nakajima, H., Fukui, H., Kelsh, R. N. and Mochizuki, N. (2016). Clarification of mural cell coverage of vascular endothelial cells by live imaging of zebrafish. *Development (Camb.)* **143**, 1328-1339. doi:10.1242/dev.132654

Ando, K., Wang, W., Peng, D., Chiba, A., Lagendijk, A. K., Barske, L., Crump, J. G., Stainier, D. Y. R., Lendahl, U., Koltowska, K. et al. (2019). Peri-arterial specification of vascular mural cells from naive mesenchyme requires Notch signaling. *Development* **146**, dev165589. doi:10.1242/dev.165589

Antonelli-Orlidge, A., Saunders, K. B., Smith, S. R. and D'Amore, P. A. (1989). An activated form of transforming growth factor β is produced by cocultures of endothelial cells and pericytes. *Proc. Natl. Acad. Sci. USA* **86**, 4544-4548. doi:10.1073/pnas.86.12.4544

Armulik, A., Abramsson, A. and Betsholtz, C. (2005). Endothelial/pericyte interactions. *Circ. Res.* **97**, 512-523. doi:10.1161/01.RES.0000182903.16652.d7

Armulik, A., Genové, G., Mäe, M., Nisancioglu, M. H., Wallgard, E., Niaudet, C., He, L., Norlin, J., Lindblom, P., Strittmatter, K. et al. (2010). Pericytes regulate the blood-brain barrier. *Nature* **468**, 557-561. doi:10.1038/nature09522

Armulik, A., Genove, G. and Betsholtz, C. (2011). Pericytes: developmental, physiological, and pathological perspectives, problems, and promises. *Dev. Cell* **21**, 193-215. doi:10.1016/j.devcel.2011.07.001

Asahina, K., Zhou, B., Pu, W. T. and Tsukamoto, H. (2011). Septum transversum-derived mesothelium gives rise to hepatic stellate cells and perivascular mesenchymal cells in developing mouse liver. *Hepatology* **53**, 983-995. doi:10.1002/hep.24119

Attwell, D., Mishra, A., Hall, C. N., O'Farrell, F. M. and Dalkara, T. (2016). What is a pericyte? *J. Cereb. Blood Flow Metab.* **36**, 451-455. doi:10.1177/0271678X15610340

Badimon, L. and Borrell-Pages, M. (2017). Wnt signaling in the vessel wall. *Curr. Opin Hematol.* **24**, 230-239. doi:10.1097/MOH.0000000000000336

Badri, K. R., Zhou, Y. and Schuger, L. (2008). Embryological origin of airway smooth muscle. *Proc. Am. Thorac. Soc.* **5**, 4-10. doi:10.1513/pats.200704-049VS

Basatemur, G. L., Jørgensen, H. F., Clarke, M. C. H., Bennett, M. R. and Mallat, Z. (2019). Vascular smooth muscle cells in atherosclerosis. *Nat. Rev. Cardiol* **16**, 727-744. doi:10.1038/s41569-019-0227-9

Boettger, T., Beetz, N., Kostin, S., Schneider, J., Kruger, M., Hein, L. and Braun, T. (2009). Acquisition of the contractile phenotype by murine arterial smooth muscle cells depends on the Mir143/145 gene cluster. *J. Clin. Invest.* **119**, 2634-2647. doi:10.1172/JCI38864

Bookman, D. E., Redmond, M. E., Waldo, K., Davis, H. and Kirby, M. L. (1987). Effect of neural crest ablation on development of the heart and arch arteries in the chick. *Am. J. Anat.* **180**, 332-341. doi:10.1002/aja.1001800403

Browning, C. L., Culberson, D. E., Aragon, I. V., Fillmore, R. A., Croissant, J. D., Schwartz, R. J. and Zimmer, W. E. (1998). The developmentally regulated expression of serum response factor plays a key role in the control of smooth muscle-specific genes. *Dev. Biol.* **194**, 18-37. doi:10.1006/dbio.1997.8808

Burnstock, G. (1970). Structure of smooth muscle and its innervation. *Smooth Muscle* Chapter 1, 1-69.

Cano, E., Carmona, R. and Munoz-Chapuli, R. (2013). Wt1-expressing progenitors contribute to multiple tissues in the developing lung. *Am. J. Physiol. Lung Cell. Mol. Physiol.* **305**, L322-L332. doi:10.1152/ajplung.00424.2012

Carmeliet, P. (2000). Mechanisms of angiogenesis and arteriogenesis. *Nat. Med.* **6**, 389-395. doi:10.1038/74651

Chen, S. and Lechleider, R. J. (2004). Transforming growth factor-β-induced differentiation of smooth muscle from a neural crest stem cell line. *Circ. Res.* **94**, 1195-1202. doi:10.1161/01.RES.0000126897.41658.81

Chen, J., Chen, H., Sanders, K. M. and Perrino, B. A. (2008). Regulation of SRF/CArG-dependent gene transcription during chronic partial obstruction of murine small intestine. *Neurogastroenterol. Motil.* **20**, 829-842. doi:10.1111/j.1365-2982.2008.01149.x

Chen, Q., Zhang, H., Liu, Y., Adams, S., Eilken, H., Stehling, M., Corada, M., Dejana, E., Zhou, B. and Adams, R. H. (2016). Endothelial cells are progenitors of cardiac pericytes and vascular smooth muscle cells. *Nat. Commun.* **7**, 1-13. doi:10.1038/ncomms12422

Chen, J., Luo, Y., Hui, H., Cai, T., Huang, H., Yang, F., Feng, J., Zhang, J. and Yan, X. (2017a). CD146 coordinates brain endothelial cell–pericyte communication for blood–brain barrier development. *Proc. Natl Acad. Sci. USA* **114**, E7622-E7631. doi:10.1073/pnas.1710848114

Chen, X., Gays, D., Milia, C. and Santoro, M. M. (2017b). Cilia control vascular mural cell recruitment in vertebrates. *Cell Reports* **18**, 1033-1047. doi:10.1016/j.celrep.2016.12.044

Cheung, C., Bernardo, A. S., Trotter, M. W., Pedersen, R. A. and Sinha, S. (2012). Generation of human vascular smooth muscle subtypes provides insight into embryological origin-dependent disease susceptibility. *Nat. Biotechnol.* **30**, 165-173. doi:10.1038/nbt.2107

Chevalier, N. R. (2018). The first digestive movements in the embryo are mediated by mechanosensitive smooth muscle calcium waves. *Phil. Trans. R. Soc. B* **373**, 20170322. doi:10.1098/rstb.2017.0322

Chevalier, N. R., Fleury, V., Dufour, S., Proux-Gillardeaux, V. and Asnacios, A. (2017). Emergence and development of gut motility in the chicken embryo. *PLoS ONE* **12**, e0172511. doi:10.1371/journal.pone.0172511

Chevalier, N. R., Ammouche, Y., Gomis, A., Teyssaire, C., de Santa Barbara, P. and Faure, S. (2020). Shifting into high gear: how interstitial cells of Cajal change the motility pattern of the developing intestine. *Am. J. Physiol. Gastrointest. Liver Physiol.* **319**, G519-G528. doi:10.1152/ajpgi.00112.2020

Climent, M., Quintavalle, M., Miragoli, M., Chen, J., Condorelli, G. and Elia, L. (2015). TGFbeta Triggers miR-143/145 Transfer From Smooth Muscle Cells to Endothelial Cells, Thereby Modulating Vessel Stabilization. *Circ. Res.* **116**, 1753-1764. doi:10.1161/CIRCRESAHA.116.305178

Cohen, E. D., Ihida-Stansbury, K., Lu, M. M., Panettieri, R. A., Jones, P. L. and Morrisey, E. E. (2009). Wnt signaling regulates smooth muscle precursor development in the mouse lung via a tenascin C/PDGFR pathway. *J. Clin. Investig.* **119**, 2538-2549. doi:10.1172/JCI38079

Cordes, K. R., Sheehy, N. T., White, M. P., Berry, E. C., Morton, S. U., Muth, A. N., Lee, T. H., Miano, J. M., Ivey, K. N. and Srivastava, D. (2009). miR-145 and miR-143 regulate smooth muscle cell fate and plasticity. *Nature* **460**, 705-710. doi:10.1038/nature08195

Cotton, J. L., Li, Q., Ma, L., Park, J. S., Wang, J., Ou, J., Zhu, L. J., Ip, Y. T., Johnson, R. L. and Mao, J. (2017). YAP/TAZ and hedgehog coordinate growth and patterning in gastrointestinal mesenchyme. *Dev. Cell* **43**, 35-47.e34. doi:10.1016/j.devcel.2017.08.019

Daneman, R., Zhou, L., Kebede, A. A. and Barres, B. A. (2010). Pericytes are required for blood-brain barrier integrity during embryogenesis. *Nature* **468**, 562-566. doi:10.1038/nature09513

del Moral, P. M., De Langhe, S. P., Sala, F. G., Veltmaat, J. M., Tefft, D., Wang, K., Warburton, D. and Bellusci, S. (2006). Differential role of FGF9 on epithelium and mesenchyme in mouse embryonic lung. *Dev. Biol.* **293**, 77-89. doi:10.1016/j.ydbio.2006.01.020

DeRuiter, M. C., Poelmann, R. E., VanMunsteren, J. C., Mironov, V., Markwald, R. R. and Gittenberger-de Groot, A. C. (1997). Embryonic endothelial cells transdifferentiate into mesenchymal cells expressing smooth muscle actins in vivo and in vitro. *Circ. Res.* **80**, 444-451. doi:10.1161/01.RES.80.4.444

de Santa Barbara, P., Williams, J., Goldstein, A., Am, D., Nielsen, C., Winfield, S., Faure, S. and Dj, R. (2005). BMPsignaling pathway plays multiple roles during gastrointestinal, tract development.

Dixit, R., Ai, X. and Fine, A. (2013). Derivation of lung mesenchymal lineages from the fetal mesothelium requires hedgehog signaling for mesothelial cell entry. *Development (Camb.)* **140**, 4398-4406. doi:10.1242/dev.098079

Dobnikar, L., Taylor, A. L., Chappell, J., Oldach, P., Harman, J. L., Oerton, E., Dzierzak, E., Bennett, M. R., Spivakov, M. and Jørgensen, H. F. (2018). Disease-relevant transcriptional signatures identified in individual smooth muscle cells from healthy mouse vessels. *Nat. Commun.* **9**, 4567. doi:10.1038/s41467-018-06891-x

Döring, Y., Pawig, L., Weber, C. and Noels, H. (2014). The CXCL12/CXCR4 chemokine ligand/receptor axis in cardiovascular disease. *Front Physiol* **5**, 212. doi:10.3389/fphys.2014.00212

Dupont, S., Morsut, L., Aragona, M., Enzo, E., Giulitti, S., Cordenonsi, M., Zanconato, F., Le Digabel, J., Forcato, M., Bicciato, S. et al. (2011). Role of YAP/TAZ in mechanotransduction. *Nature* **474**, 179-183. doi:10.1038/nature10137

El Agha, E., Herold, S., Al Alam, D., Quantius, J., MacKenzie, B., Carraro, G., Moiseenko, A., Chao, C. M., Minoo, P., Seeger, W. et al. (2014). Fgf10-positive cells represent a progenitor cell population during lung development and postnatally. *Development* **141**, 296-306. doi:10.1242/dev.099747

Etchevers, H. C., Vincent, C., Le Douarin, N. M. and Couly, G. F. (2001). The cephalic neural crest provides pericytes and smooth muscle cells to all blood vessels of the face and forebrain. *Development* **128**, 1059-1068.

Ezratty, E. J., Stokes, N., Chai, S., Shah, A. S., Williams, S. E. and Fuchs, E. (2011). A role for the primary cilium in notch signaling and epidermal differentiation during skin development. *Cell* **145**, 1129-1141. doi:10.1016/j.cell.2011.05.030

Farmer, S. G., Hay, D. W., Raeburn, D. and Fedan, J. S. (1987). Relaxation of guinea-pig tracheal smooth muscle to arachidonate is converted to contraction following epithelium removal. *Br. J. Pharmacol.* **92**, 231-236. doi:10.1111/j.1476-5381.1987.tb11316.x

Forstermann, U. and Sessa, W. C. (2012). Nitric oxide synthases: regulation and function. *Eur Heart J* **33**, 829-837, 837a-837d. doi:10.1093/eurheartj/ehr304

Fortuna, V., Pardanaud, L., Brunet, I., Ola, R., Ristori, E., Santoro, M. M., Nicoli, S. and Eichmann, A. (2015). Vascular mural cells promote noradrenergic

differentiation of embryonic sympathetic neurons. *Cell Reports* **11**, 1786-1796. doi:10.1016/j.celrep.2015.05.028
Foster, K., Sheridan, J., Veiga-Fernandes, H., Roderick, K., Pachnis, V., Adams, R., Blackburn, C., Kioussis, D. and Coles, M. (2008). Contribution of neural crest-derived cells in the embryonic and adult thymus. *J. Immunol.* **180**, 3183-3189. doi:10.4049/jimmunol.180.5.3183
Furchgott, R. F. and Vanhoutte, P. M. (1989). Endothelium-derived relaxing and contracting factors. *FASEB J.* **3**, 2007-2018. doi:10.1096/fasebj.3.9.2545495
Gabella, G. (1981). Structure of smooth muscle. In *Smooth Muscle, An Assessment of Current Knowledge* (E. Bülbring, A. F. Brading, A. W. Jones and T. Tomita, eds), pp. 1-46. London: Edward Arnold.
Gabella, G. (1990). Hypertrophy of visceral smooth muscle. *Anat Embryol (Berl)* **182**, 409-424. doi:10.1007/BF00178906
Gabella, G. (2002). Development of visceral smooth muscle. *Results Probl. Cell Differ.* **38**, 1-37. doi:10.1007/978-3-540-45686-5_1
Gaengel, K., Genove, G., Armulik, A. and Betsholtz, C. (2009). Endothelial-mural cell signaling in vascular development and angiogenesis. *Arterioscler. Thromb. Vasc. Biol.* **29**, 630-638. doi:10.1161/ATVBAHA.107.161521
Gays, D., Hess, C., Camporeale, A., Ala, U., Provero, P., Mosimann, C. and Santoro, M. M. (2017). An exclusive cellular and molecular network governs intestinal smooth muscle cell differentiation in vertebrates. *Development (Camb.)* **144**, 464-478. doi:10.1242/dev.133926
Georgijevic, S., Subramanian, Y., Rollins, E. L., Starovic-Subota, O., Tang, A. C. and Childs, S. J. (2007). Spatiotemporal expression of smooth muscle markers in developing zebrafish gut. *Dev. Dyn.* **236**, 1623-1632. doi:10.1002/dvdy.21165
Gillman, T. A. and Pennefather, J. N. (1998). Evidence for the presence of both P1 and P2 purinoceptors in the rat myometrium. *Clin. Exp. Pharmacol. Physiol.* **25**, 592-599. doi:10.1111/j.1440-1681.1998.tb02257.x
Gittenberger-de Groot, A. C., Vrancken Peeters, M.-P. F. M., Mentink, M. M. T., Gourdie, R. G. and Poelmann, R. E. (1998). Epicardium-derived cells contribute a novel population to the myocardial wall and the atrioventricular cushions. *Circ. Res.* **82**, 1043-1052. doi:10.1161/01.RES.82.10.1043
Goodwin, K., Mao, S., Guyomar, T., Miller, E., Radisky, D. C., Kosmrlj, A. and Nelson, C. M. (2019). Smooth muscle differentiation shapes domain branches during mouse lung development. *Development* **146**, dev181172. doi:10.1242/dev.181172
Goumans, M. J. and Ten Dijke, P. (2018). TGF-β Signaling in control of cardiovascular function. *Cold Spring Harb Perspect Biol* **10**. doi:10.1101/cshperspect.a022210
Grainger, D. J., Metcalfe, J. C., Grace, A. A. and Mosedale, D. E. (1998). Transforming growth factor-β dynamically regulates vascular smooth muscle differentiation in vivo. *J. Cell Sci.* **111**, 2977-2988.
Halayko, A. J., Salari, H., Ma, X. and Stephens, N. L. (1996). Markers of airway smooth muscle cell phenotype. *Am. J. Physiol.* **270**, L1040-L1051. doi:10.1152/ajplung.1996.270.6.L1040
Hall, C. N., Reynell, C., Gesslein, B., Hamilton, N. B., Mishra, A., Sutherland, B. A., O'Farrell, F. M., Buchan, A. M., Lauritzen, M. and Attwell, D. (2014). Capillary pericytes regulate cerebral blood flow in health and disease. *Nature* **508**, 55-60. doi:10.1038/nature13165
Hartmann, D. A., Underly, R. G., Grant, R. I., Watson, A. N., Lindner, V. and Shih, A. Y. (2015). Pericyte structure and distribution in the cerebral cortex revealed by high-resolution imaging of transgenic mice. *Neurophotonics* **2**, 041402. doi:10.1117/1.NPh.2.4.041402
Hellstrom, M., Kalen, M., Lindahl, P., Abramsson, A. and Betsholtz, C. (1999). Role of PDGF-B and PDGFR-beta in recruitment of vascular smooth muscle cells and pericytes during embryonic blood vessel formation in the mouse. *Development* **126**, 3047-3055.
Herbert, S. P. and Stainier, D. Y. (2011). Molecular control of endothelial cell behaviour during blood vessel morphogenesis. *Nat. Rev. Mol. Cell Biol.* **12**, 551-564. doi:10.1038/nrm3176
Hergenreider, E., Heydt, S., Tréguer, K., Boettger, T., Horrevoets, A. J. G., Zeiher, A. M., Scheffer, M. P., Frangakis, A. S., Yin, X., Mayr, M. et al. (2012). Atheroprotective communication between endothelial cells and smooth muscle cells through miRNAs. *Nat. Cell Biol.* **14**, 249-256. doi:10.1038/ncb2441
Herriges, J. C., Verheyden, J. M., Zhang, Z., Sui, P., Zhang, Y., Anderson, M. J., Swing, D. A., Zhang, Y., Lewandoski, M. and Sun, X. (2015). FGF-Regulated ETV transcription factors control FGF-SHH feedback loop in lung branching. *Dev. Cell* **35**, 322-332. doi:10.1016/j.devcel.2015.10.006
Hill-Eubanks, D. C., Werner, M. E., Heppner, T. J. and Nelson, M. T. (2011). Calcium signaling in smooth muscle. *Cold Spring Harb. Perspect Biol.* **3**, a004549. doi:10.1101/cshperspect.a004549
Horne-Badovinac, S., Rebagliati, M. and Stainier, D. Y. (2003). A cellular framework for gut-looping morphogenesis in zebrafish. *Science* **302**, 662-665. doi:10.1126/science.1085397
Huddleson, J. P., Srinivasan, S., Ahmad, N. and Lingrel, J. B. (2004). Fluid shear stress induces endothelial KLF2 gene expression through a defined promoter region. *Biol. Chem.* **385**, 723-729. doi:10.1515/BC.2004.088
Huycke, T. R., Miller, B. M., Gill, H. K., Nerurkar, N. L., Sprinzak, D., Mahadevan, L. and Tabin, C. J. (2019). Genetic and mechanical regulation of intestinal smooth muscle development. *Cell* **179**, 90-105.e121. doi:10.1016/j.cell.2019.08.041
Iivanainen, E., Nelimarkka, L., Elenius, V., Heikkinen, S.-M., Junttila, T. T., Sihombing, L., Sundvall, M., Määttä, J. A., Laine, V. J. O., Ylä-Herttuala, S. et al. (2003). Angiopoietin-regulated recruitment of vascular smooth muscle cells by endothelial-derived heparin binding EGF-like growth factor. *FASEB J.* **17**, 1609-1621. doi:10.1096/fj.02-0939com
Jahoda, C. A., Reynolds, A. J., Chaponnier, C., Forester, J. C. and Gabbiani, G. (1991). Smooth muscle alpha-actin is a marker for hair follicle dermis in vivo and in vitro. *J. Cell Sci.* **99**, 627-636.
Jakab, M. and Augustin, H. G. (2020). Understanding angiodiversity: insights from single cell biology. *Development* **147**, dev146621. doi:10.1242/dev.146621
Jakkaraju, S., Zhe, X., Pan, D., Choudhury, R. and Schuger, L. (2005). TIPs are tension-responsive proteins involved in myogenic versus adipogenic differentiation. *Dev. Cell* **9**, 39-49. doi:10.1016/j.devcel.2005.04.015
Jesudason, E. C. (2009). Airway smooth muscle: an architect of the lung? *Thorax* **64**, 541-545. doi:10.1136/thx.2008.107094
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.
Kedinger, M., Simon-Assmann, P., Bouziges, F., Arnold, C., Alexandra, E. and Haffen, K. (1990). Smooth muscle actin expression during rat gut development and induction in fetal skin fibroblastic cells associated with intestinal embryonic epithelium. *Differentiation* **43**, 87-97. doi:10.1111/j.1432-0436.1990.tb00434.x
Kelley, C., Clements, W., Glenn, N. and Santoro, M. (2019). Contribution of sclerotome to the embryonic hematopoietic stem cell niche and vascular smooth muscle. *Exp. Hematol.* **76**, S71. doi:10.1016/j.exphem.2019.06.377
Khalipina, D., Kaga, Y., Dacher, N. and Chevalier, N. R. (2019). Smooth muscle contractility causes the gut to grow anisotropically. *J. R Soc. Interface* **16**, 20190484. doi:10.1098/rsif.2019.0484
Kim, B. M. and Choi, M. Y. (2009). New insights into the role of hedgehog signaling in gastrointestinal development and cancer. *Gastroenterology* **137**, 422-424. doi:10.1053/j.gastro.2009.06.021
Kim, H. Y., Pang, M.-F., Varner, V. D., Kojima, L., Miller, E., Radisky, D. C. and Nelson, C. M. (2015). Localized smooth muscle differentiation is essential for epithelial bifurcation during branching morphogenesis of the mammalian lung. *Dev. Cell* **34**, 719-726. doi:10.1016/j.devcel.2015.08.012
Kirby, M. L. (1989). Plasticity and predetermination of mesencephalic and trunk neural crest transplanted into the region of the cardiac neural crest. *Dev. Biol.* **134**, 402-412. doi:10.1016/0012-1606(89)90112-7
Korff, T., Kimmina, S., Martiny-Baron, G. and Augustin, H. G. (2001). Blood vessel maturation in a 3-dimensional spheroidal coculture model: direct contact with smooth muscle cells regulates endothelial cell quiescence and abrogates VEGF responsiveness. *FASEB J.* **15**, 447-457. doi:10.1096/fj.00-0139com
Kulesa, P. M. and Fraser, S. E. (2000). In ovo time-lapse analysis of chick hindbrain neural crest cell migration shows cell interactions during migration to the branchial arches. *Development* **127**, 1161-1172.
Kumar, M. E., Bogard, P. E., Espinoza, F. H., Menke, D. B., Kingsley, D. M. and Krasnow, M. A. (2014). Defining a mesenchymal progenitor niche at single-cell resolution. *Science* **346**, 1258810. doi:10.1126/science.1258810
Le Guen, L., Marchal, S., Faure, S. and de Santa Barbara, P. (2015). Mesenchymal-epithelial interactions during digestive tract development and epithelial stem cell regeneration. *Cell. Mol. Life Sci.* **72**, 3883-3896. doi:10.1007/s00018-015-1975-2
Le Lièvre, C. S. and Le Douarin, N. M. (1975). Mesenchymal derivatives of the neural crest: analysis of chimaeric quail and chick embryos. *Development* **34**, 125-115.
Lee, J. H., Tammela, T., Hofree, M., Choi, J., Marjanovic, N. D., Han, S., Canner, D., Wu, K., Paschini, M., Bhang, D. H. et al. (2017). Anatomically and functionally distinct lung mesenchymal populations marked by Lgr5 and Lgr6. *Cell* **170**, 1149-1163.e1112. doi:10.1016/j.cell.2017.07.028
Leeper, N. J., Raiesdana, A., Kojima, Y., Chun, H. J., Azuma, J., Maegdefessel, L., Kundu, R. K., Quertermous, T., Tsao, P. S. and Spin, J. M. (2011). MicroRNA-26a is a novel regulator of vascular smooth muscle cell function. *J. Cell. Physiol.* **226**, 1035-1043. doi:10.1002/jcp.22422
Leitch, C. C., Lodh, S., Prieto-Echagüe, V., Badano, J. L. and Zaghloul, N. A. (2014). Basal body proteins regulate Notch signaling through endosomal trafficking. *J. Cell Sci.* **127**, 2407-2419. doi:10.1242/jcs.130344
Li, W., Kohara, H., Uchida, Y., James, J. M., Soneji, K., Cronshaw, D. G., Zou, Y.-R., Nagasawa, T. and Mukouyama, Y.-S. (2013). Peripheral nerve-derived CXCL12 and VEGF-A regulate the patterning of arterial vessel branching in developing limb skin. *Dev. Cell* **24**, 359-371. doi:10.1016/j.devcel.2013.01.009
Lin, Y. M., Li, F. and Shi, X. Z. (2012). Mechano-transcription of COX-2 is a common response to lumen dilation of the rat gastrointestinal tract. *Neurogastroenterol. Motil.* **24**, 670-677, e295-676. doi:10.1111/j.1365-2982.2012.01918.x
Lindahl, P., Johansson, B. R., Levéen, P. and Betsholtz, C. (1997a). Pericyte loss and microaneurysm formation in PDGF-B-deficient mice. *Science* **277**, 242-245. doi:10.1126/science.277.5323.242
Lindahl, P., Karlsson, L., Hellström, M., Gebre-Medhin, S., Willetts, K., Heath, J. K. and Betsholtz, C. (1997b). Alveogenesis failure in PDGF-A-deficient mice is

coupled to lack of distal spreading of alveolar smooth muscle cell progenitors during lung development. *Development* **124**, 3943-3953.
Liu, X., Chen, W., Li, W., Li, Y., Priest, J. R., Zhou, B., Wang, J. and Zhou, Z. (2019). Single-cell RNA-Seq of the developing cardiac outflow tract reveals convergent development of the vascular smooth muscle cells. *Cell Rep* **28**, 1346-1361.e1344. doi:10.1016/j.celrep.2019.06.092
Low, R. B. and White, S. L. (1998). Lung smooth muscle differentiation. *Int. J. Biochem. Cell Biol.* **30**, 869-883. doi:10.1016/S1357-2725(98)00049-1
Lumsden, A., Sprawson, N. and Graham, A. (1991). Segmental origin and migration of neural crest cells in the hindbrain region of the chick embryo. *Development* **113**, 1281-1291.
MacDonald, J. A. (2008). Smooth muscle phenotypic plasticity in mechanical obstruction of the small intestine. *Neurogastroenterol. Motil.* **20**, 737-740. doi:10.1111/j.1365-2982.2008.01148.x
Mailleux, A. A., Kelly, R., Veltmaat, J. M., De Langhe, S. P., Zaffran, S., Thiery, J. P. and Bellusci, S. (2005). Fgf10 expression identifies parabronchial smooth muscle cell progenitors and is required for their entry into the smooth muscle cell lineage. *Development* **132**, 2157-2166. doi:10.1242/dev.01795
Majesky, M. W. (2007). Developmental basis of vascular smooth muscle diversity. *Arterioscler. Thromb. Vasc. Biol.* **27**, 1248-1258. doi:10.1161/ATVBAHA.107.141069
Mao, J., Kim, B.-M., Rajurkar, M., Shivdasani, R. A. and McMahon, A. P. (2010). Hedgehog signaling controls mesenchymal growth in the developing mammalian digestive tract. *Development* **137**, 1721-1729. doi:10.1242/dev.044586
McCray, P. B. Jr. (1993). Spontaneous contractility of human fetal airway smooth muscle. *Am. J. Respir. Cell Mol. Biol.* **8**, 573-580. doi:10.1165/ajrcmb/8.5.573
McHugh, K. M. (1995). Molecular analysis of smooth muscle development in the mouse. *Dev. Dyn.* **204**, 278-290. doi:10.1002/aja.1002040306
McHugh, K. M. (1996). Molecular analysis of gastrointestinal smooth muscle development. *J. Pediatr. Gastroenterol. Nutr.* **23**, 379-394. doi:10.1097/00005176-199611000-00001
Mellgren, A. M., Smith, C. L., Olsen, G. S., Eskiocak, B., Zhou, B., Kazi, M. N., Ruiz, F. R., Pu, W. T. and Tallquist, M. D. (2008). Platelet-derived growth factor receptor beta signaling iMellgren, A. M., Smith, C. L., Olsen, G. S., Eskiocak, B., Zhou, B., Kazi, M. N., … Tallquist, M. D. (2008). Platelet-derived growth factor receptor beta signaling is required for efficient epicardia. *Circ. Res.* **103**, 1393-1401. doi:10.1161/CIRCRESAHA.108.176768
Mikawa, T. and Gourdie, R. G. (1996). Pericardial mesoderm generates a population of coronary smooth muscle cells migrating into the heart along with ingrowth of the epicardial organ. *Dev. Biol.* **174**, 221-232. doi:10.1006/dbio.1996.0068
Miller, L.-A. D., Wert, S. E., Clark, J. C., Xu, Y., Perl, A.-K. T. and Whitsett, J. A. (2004). Role of Sonic hedgehog in patterning of tracheal-bronchial cartilage and the peripheral lung. *Dev. Dyn.* **231**, 57-71. doi:10.1002/dvdy.20105
Miralles, F., Posern, G., Zaromytidou, A. I. and Treisman, R. (2003). Actin dynamics control SRF activity by regulation of its coactivator MAL. *Cell* **113**, 329-342. doi:10.1016/S0092-8674(03)00278-2
Mitchell, T. S., Bradley, J., Robinson, G. S., Shima, D. T. and Ng, Y. S. (2008). RGS5 expression is a quantitative measure of pericyte coverage of blood vessels. *Angiogenesis* **11**, 141-151. doi:10.1007/s10456-007-9085-x
Moiseenko, A., Kheirollahi, V., Chao, C. M., Ahmadvand, N., Quantius, J., Wilhelm, J., Herold, S., Ahlbrecht, K., Morty, R. E., Rizvanov, A. A. et al. (2017). Origin and characterization of alpha smooth muscle actin-positive cells during murine lung development. *Stem Cells* **35**, 1566-1578. doi:10.1002/stem.2615
Moya, I. M. and Halder, G. (2019). Hippo-YAP/TAZ signalling in organ regeneration and regenerative medicine. *Nat. Rev. Mol. Cell Biol.* **20**, 211-226. doi:10.1038/s41580-018-0086-y
Muhl, L., Genové, G., Leptidis, S., Liu, J., He, L., Mocci, G., Sun, Y., Gustafsson, S., Buyandelger, B., Chivukula, I. V. et al. (2020). Single-cell analysis uncovers fibroblast heterogeneity and criteria for fibroblast and mural cell identification and discrimination. *Nat. Commun.* **11**, 3953. doi:10.1038/s41467-020-17740-1
Nakano, H., Williams, E., Hoshijima, M., Sasaki, M., Minamisawa, S., Chien, K. R. and Nakano, A. (2011). Cardiac origin of smooth muscle cells in the inflow tract. *J. Mol. Cell. Cardiol.* **50**, 337-345. doi:10.1016/j.yjmcc.2010.10.009
Okpalaugo, E. O., Garcez-do-Carmo, L., Jurkiewicz, N. H. and Jurkiewicz, A. (2002). Contractile responses of the rat vas deferens after epithelium removal. *Life Sci.* **70**, 2943-2951. doi:10.1016/S0024-3205(02)01545-X
Olson, E. N. and Nordheim, A. (2010). Linking actin dynamics and gene transcription to drive cellular motile functions. *Nat. Rev. Mol. Cell Biol.* **11**, 353-365. doi:10.1038/nrm2890
Olson, L. E. and Soriano, P. (2011). PDGFRbeta signaling regulates mural cell plasticity and inhibits fat development. *Dev. Cell* **20**, 815-826. doi:10.1016/j.devcel.2011.04.019
Owens, G. K., Kumar, M. S. and Wamhoff, B. R. (2004). Molecular regulation of vascular smooth muscle cell differentiation in development and disease. *Physiol. Rev.* **84**, 767-801. doi:10.1152/physrev.00041.2003
Ozerdem, U., Grako, K. A., Dahlin-Huppe, K., Monosov, E. and Stallcup, W. B. (2001). NG2 proteoglycan is expressed exclusively by mural cells during vascular morphogenesis. *Dev. Dyn.* **222**, 218-227. doi:10.1002/dvdy.1200
Parvez, O., Voss, A. M., de Kok, M., Roth-Kleiner, M. and Belik, J. (2006). Bronchial muscle peristaltic activity in the fetal rat. *Pediatr. Res.* **59**, 756-761. doi:10.1203/01.pdr.0000219121.15634.d1
Pepicelli, C. V., Lewis, P. M. and McMahon, A. P. (1998). Sonic hedgehog regulates branching morphogenesis in the mammalian lung. *Curr. Biol.* **8**, 1083-1086. doi:10.1016/S0960-9822(98)70446-4
Pouget, C., Gautier, R., Teillet, M. A. and Jaffredo, T. (2006). Somite-derived cells replace ventral aortic hemangioblasts and provide aortic smooth muscle cells of the trunk. *Development* **133**, 1013-1022. doi:10.1242/dev.02269
Prummel, K. D., Nieuwenhuize, S. and Mosimann, C. (2020). The lateral plate mesoderm. *Development* **147**, dev175059. doi:10.1242/dev.175059
Qi, Y.-X., Yao, Q.-P., Huang, K., Shi, Q., Zhang, P., Wang, G.-L., Han, Y., Bao, H., Wang, L., Li, H.-P. et al. (2016). Nuclear envelope proteins modulate proliferation of vascular smooth muscle cells during cyclic stretch application. *Proc. Natl Acad. Sci. USA* **113**, 5293-5298. doi:10.1073/pnas.1604569113
Que, J., Wilm, B., Hasegawa, H., Wang, F., Bader, D. and Hogan, B. L. (2008). Mesothelium contributes to vascular smooth muscle and mesenchyme during lung development. *Proc. Natl. Acad. Sci. USA* **105**, 16626-16630. doi:10.1073/pnas.0808649105
Ramalho-Santos, M., Melton, D. A. and McMahon, A. P. (2000). Hedgehog signals regulate multiple aspects of gastrointestinal development. *Development* **127**, 2763-2772.
Ramasamy, S. K., Mailleux, A. A., Gupte, V. V., Mata, F., Sala, F. G., Veltmaat, J. M., Del Moral, P. M., De Langhe, S., Parsa, S., Kelly, L. K. et al. (2007). Fgf10 dosage is critical for the amplification of epithelial cell progenitors and for the formation of multiple mesenchymal lineages during lung development. *Dev. Biol.* **307**, 237-247. doi:10.1016/j.ydbio.2007.04.033
Roberts, D. J., Smith, D. M., Goff, D. J. and Tabin, C. J. (1998). Epithelial-mesenchymal signaling during the regionalization of the chick gut. *Development* **125**, 2791-2801.
Santoro, M. M., Pesce, G. and Stainier, D. Y. (2009). Characterization of vascular mural cells during zebrafish development. *Mech. Dev.* **126**, 638-649. doi:10.1016/j.mod.2009.06.1080
Sato, Y. and Rifkin, D. B. (1989). Inhibition of endothelial cell movement by pericytes and smooth muscle cells: Activation of a latent transforming growth factor-β1-like molecule by plasmin during co-culture. *J. Cell Biol.* **109**, 309-315. doi:10.1083/jcb.109.1.309
Sawada, H., Rateri, D. L., Moorleghen, J. J., Majesky, M. W. and Daugherty, A. (2017). Smooth muscle cells derived from second heart field and cardiac neural crest reside in spatially distinct domains in the media of the ascending aorta - brief report. *Arterioscler. Thromb. Vasc. Biol.* **37**, 1722-1726. doi:10.1161/ATVBAHA.117.309599
Sedding, D. G., Seay, U., Fink, L., Heil, M., Kummer, W., Tillmanns, H. and Braun-Dullaeus, R. C. (2003). Mechanosensitive p27Kip1 regulation and cell cycle entry in vascular smooth muscle cells. *Circulation* **108**, 616-622. doi:10.1161/01.CIR.0000079102.08464.E2
Shan, L., Subramaniam, M., Emanuel, R. L., Degan, S., Johnston, P., Tefft, D., Warburton, D. and Sunday, M. E. (2008). Centrifugal migration of mesenchymal cells in embryonic lung. *Dev. Dyn.* **237**, 750-757. doi:10.1002/dvdy.21462
Shi, X. Z. (2017). Mechanical regulation of gene expression in gut smooth muscle cells. *Front Physiol* **8**, 1000. doi:10.3389/fphys.2017.01000
Shimada, T., Fabian, M., Yan, H. Q. and Nishimura, H. (1998). Control of vascular smooth muscle cell growth in fowl. *Gen. Comp. Endocrinol.* **112**, 115-128. doi:10.1006/gcen.1998.7150
Shyer, A. E., Tallinen, T., Nerurkar, N. L., Wei, Z., Gil, E. S., Kaplan, D. L., Tabin, C. J. and Mahadevan, L. (2013). Villification: How the gut gets its villi. *Science* **342**, 212-218. doi:10.1126/science.1238842
Sims, D. E. (1986). *The Pericyte-A Review*, pp. 153-174: Churchill Livingstone.
Sinha, S. and Santoro, M. M. (2018). New models to study vascular mural cell embryonic origin: implications in vascular diseases. *Cardiovasc. Res.* **114**, 481-491. doi:10.1093/cvr/cvy005
Smith, C. L., Baek, S. T., Sung, C. Y. and Tallquist, M. D. (2011). Epicardial-derived cell epithelial-to-mesenchymal transition and fate specification require PDGF receptor signaling. *Circ. Res.* **108**, e15-e26. doi:10.1161/CIRCRESAHA.110.235531
Snitow, M., Lu, M., Cheng, L., Zhou, S. and Morrisey, E. E. (2016). Ezh2 restricts the smooth muscle lineage during mouse lung mesothelial development. *Development* **143**, 3733-3741. doi:10.1242/dev.134932
Song, N., Huang, Y., Shi, H., Yuan, S., Ding, Y., Song, X., Fu, Y. and Luo, Y. (2009). Overexpression of platelet-derived growth factor-BB increases tumor pericyte content via stromal-derived factor-1alpha/CXCR4 axis. *Cancer Res.* **69**, 6057-6064. doi:10.1158/0008-5472.CAN-08-2007
Sparrow, M. P. and Lamb, J. P. (2003). Ontogeny of airway smooth muscle: structure, innervation, myogenesis and function in the fetal lung. *Respir. Physiol. Neurobiol.* **137**, 361-372. doi:10.1016/S1569-9048(03)00159-9
Stallcup, W. B. (2018). The NG2 proteoglycan in pericyte biology. *Adv. Exp. Med. Biol.* **1109**, 5-19. doi:10.1007/978-3-030-02601-1_2
Stephens, N. L. (2001). Airway smooth muscle. *Lung* **179**, 333-373. doi:10.1007/s004080000073

Stratman, A. N., Burns, M. C., Farrelly, O. M., Davis, A. E., Li, W., Pham, V. N., Castranova, D., Yano, J. J., Goddard, L. M., Nguyen, O. et al. (2020). Chemokine mediated signalling within arteries promotes vascular smooth muscle cell recruitment. *Commun. Biol.* **3**, 734. doi:10.1038/s42003-020-01462-7

Stratman, A. N., Pezoa, S. A., Farrelly, O. M., Castranova, D., Dye, L. E., Butler, M. G., Sidik, H., Talbot, W. S. and Weinstein, B. M. (2017). Interactions between mural cells and endothelial cells stabilize the developing zebrafish dorsal aorta. *Development (Camb.)* **144**, 115-127. doi:10.1242/dev.143131

Stratman, A. N., Schwindt, A. E., Malotte, K. M. and Davis, G. E. (2010). Endothelial-derived PDGF-BB and HB-EGF coordinately regulate pericyte recruitment during vasculogenic tube assembly and stabilization. *Blood* **116**, 4720-4730. doi:10.1182/blood-2010-05-286872

Sukegawa, A., Narita, T., Kameda, T., Saitoh, K., Nohno, T., Iba, H., Yasugi, S. and Fukuda, K. (2000). The concentric structure of the developing gut is regulated by Sonic hedgehog derived from endodermal epithelium. *Development* **127**, 1971-1980.

Turczyńska, K. M., Hellstrand, P., Swärd, K. and Albinsson, S. (2013). Regulation of vascular smooth muscle mechanotransduction by microRNAs and L-type calcium channels. *Commun. Integr. Biol.* **6**, e22278. doi:10.4161/cib.22278

van Kuijk, K., Kuppe, C., Betsholtz, C., Vanlandewijck, M., Kramann, R. and Sluimer, J. C. (2019). Heterogeneity and plasticity in healthy and atherosclerotic vasculature explored by single-cell sequencing. *Cardiovasc. Res.* **115**, 1705-1715. doi:10.1093/cvr/cvz185

Vanlandewijck, M., He, L., Mäe, M. A., Andrae, J., Ando, K., Del Gaudio, F., Nahar, K., Lebouvier, T., Laviña, B., Gouveia, L. et al. (2018). A molecular atlas of cell types and zonation in the brain vasculature. *Nature* **554**, 475-480. doi:10.1038/nature25739

Vrancken Peeters, M. P., Gittenberger-de Groot, A. C., Mentink, M. M. and Poelmann, R. E. (1999). Smooth muscle cells and fibroblasts of the coronary arteries derive from epithelial-mesenchymal transformation of the epicardium. *Anat. Embryol. (Berl)* **199**, 367-378. doi:10.1007/s004290050235

Wagenseil, J. E. and Mecham, R. P. (2009). Vascular extracellular matrix and arterial mechanics. *Physiol. Rev.* **89**, 957-989. doi:10.1152/physrev.00041.2008

Waldo, K. L., Hutson, M. R., Ward, C. C., Zdanowicz, M., Stadt, H. A., Kumiski, D., Abu-Issa, R. and Kirby, M. L. (2005). Secondary heart field contributes myocardium and smooth muscle to the arterial pole of the developing heart. *Dev. Biol.* **281**, 78-90. doi:10.1016/j.ydbio.2005.02.012

Wallace, A. S. and Burns, A. J. (2005). Development of the enteric nervous system, smooth muscle and interstitial cells of Cajal in the human gastrointestinal tract. *Cell Tissue Res.* **319**, 367-382. doi:10.1007/s00441-004-1023-2

Wallace, K. N., Akhter, S., Smith, E. M., Lorent, K. and Pack, M. (2005). Intestinal growth and differentiation in zebrafish. *Mech. Dev.* **122**, 157-173. doi:10.1016/j.mod.2004.10.009

Walton, K. D., Whidden, M., Kolterud, Å., Shoffner, S. K., Czerwinski, M. J., Kushwaha, J., Parmar, N., Chandhrasekhar, D., Freddo, A. M., Schnell, S. et al. (2016). Villification in the mouse: Bmp signals control intestinal villus patterning. *Development* **143**, 427-436. doi:10.1242/dev.130112

Wan, H., Dingle, S., Xu, Y., Besnard, V., Kaestner, K. H., Ang, S. L., Wert, S., Stahlman, M. T. and Whitsett, J. A. (2005). Compensatory roles of Foxal and Foxa2 during lung morphogenesis. *J. Biol. Chem.* **280**, 13809-13816. doi:10.1074/jbc.M414122200

Wang, G., Jacquet, L., Karamariti, E. and Xu, Q. (2015). Origin and differentiation of vascular smooth muscle cells. *J. Physiol.* **593**, 3013-3030. doi:10.1113/JP270033

Wang, Q., Zhao, N., Kennard, S. and Lilly, B. (2012). Notch2 and Notch3 Function Together to Regulate Vascular Smooth Muscle Development. *PLoS ONE* **7**, e37365-e37365.

Wang, Y. Y., Pan, L. Y., Moens, C. B. and Appel, B. (2014). Notch3 establishes brain vascular integrity by regulating pericyte number. *Development (Camb.)* **141**, 307-317. doi:10.1242/dev.096107

Wang, Z., Shu, W., Lu, M. M. and Morrisey, E. E. (2005). Wnt7b activates canonical signaling in epithelial and vascular smooth muscle cells through interactions with Fzd1, Fzd10, and LRP5. *Mol. Cell. Biol.* **25**, 5022-5030. doi:10.1128/MCB.25.12.5022-5030.2005

Wasteson, P., Johansson, B. R., Jukkola, T., Breuer, S., Akydsurek, L. M., Partanen, J. and Lindahl, P. (2008). Developmental origin of smooth muscle cells in the descending aorta in mice. *Development* **135**, 1823-1832. doi:10.1242/dev.020958

Watterston, C., Zeng, L., Onabadejo, A. and Childs, S. J. (2019). MicroRNA26 attenuates vascular smooth muscle maturation via endothelial BMP signalling. *PLoS Genet.* **15**, e1008163-e1008163. doi:10.1371/journal.pgen.1008163

Weaver, M., Batts, L. and Hogan, B. L. M. (2003). Tissue interactions pattern the mesenchyme of the embryonic mouse lung. *Dev. Biol.* **258**, 169-184. doi:10.1016/S0012-1606(03)00117-9

Whalley, E. T. (1978). The action of bradykinin and oxytocin on the isolated whole uterus and myometrium of the rat in oestrus. *Br. J. Pharmacol.* **64**, 21-28. doi:10.1111/j.1476-5381.1978.tb08636.x

Whitesell, T. R., Chrystal, P. W., Ryu, J.-R., Munsie, N., Grosse, A., French, C. R., Workentine, M. L., Li, R., Zhu, L. J., Waskiewicz, A. et al. (2019). foxc1 is required for embryonic head vascular smooth muscle differentiation in zebrafish. *Dev. Biol.* **453**, 34-47. doi:10.1016/j.ydbio.2019.06.005

Whitesell, T. R., Kennedy, R. M., Carter, A. D., Rollins, E.-L., Georgijevic, S., Santoro, M. M. and Childs, S. J. (2014). An α-Smooth muscle actin (acta2/αsma) Zebrafish transgenic line marking vascular mural cells and visceral smooth muscle cells. *PLoS ONE* **9**, e90590-e90590. doi:10.1371/journal.pone.0090590

Wiegreffe, C., Christ, B., Huang, R. and Scaal, M. (2007). Sclerotomal origin of smooth muscle cells in the wall of the avian dorsal aorta. *Dev. Dyn.* **236**, 2578-2585. doi:10.1002/dvdy.21279

Wiegreffe, C., Christ, B., Huang, R. and Scaal, M. (2009). Remodeling of aortic smooth muscle during avian embryonic development. *Dev. Dyn.* **238**, 624-631. doi:10.1002/dvdy.21888

Wilm, B., Ipenberg, A., Hastie, N. D., Burch, J. B. and Bader, D. M. (2005). The serosal mesothelium is a major source of smooth muscle cells of the gut vasculature. *Development* **132**, 5317-5328. doi:10.1242/dev.02141

Wilson, C., Lee, M. D. and McCarron, J. G. (2016). Acetylcholine released by endothelial cells facilitates flow-mediated dilatation. *J. Physiol.* **594**, 7267-7307. doi:10.1113/JP272927

Wu, J., Bohanan, C. S., Neumann, J. C. and Lingrel, J. B. (2008). KLF2 Transcription Factor Modulates Blood Vessel Maturation through Smooth Muscle Cell Migration. *J. Biol. Chem.* **283**, 3942-3950. doi:10.1074/jbc.M707882200

Yamazaki, T., Nalbandian, A., Uchida, Y., Li, W., Arnold, T. D., Kubota, Y., Yamamoto, S., Ema, M. and Mukouyama, Y. S. (2017). Tissue myeloid progenitors differentiate into Pericytes through TGF-β signaling in developing skin vasculature. *Cell Reports* **18**, 2991-3004. doi:10.1016/j.celrep.2017.02.069

Yanagisawa, M., Kurihara, H., Kimura, S., Tomobe, Y., Kobayashi, M., Mitsui, Y., Yazaki, Y., Goto, K. and Masaki, T. (1988). A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature* **332**, 411-415. doi:10.1038/332411a0

Yang, X., Dong, M., Wen, H., Liu, X., Zhang, M., Ma, L., Zhang, C., Luan, X., Lu, H. and Zhang, Y. (2017). MiR-26a contributes to the PDGF-BB-induced phenotypic switch of vascular smooth muscle cells by suppressing Smad1. *Oncotarget* **8**, 75844-75853. doi:10.18632/oncotarget.17998

Yang, Y., Beqaj, S., Kemp, P., Ariel, I. and Schuger, L. (2000). Stretch-induced alternative splicing of serum response factor promotes bronchial myogenesis and is defective in lung hypoplasia. *J. Clin. Invest.* **106**, 1321-1330. doi:10.1172/JCI8893

Yang, Y., Relan, N. K., Przywara, D. A. and Schuger, L. (1999). Embryonic mesenchymal cells share the potential for smooth muscle differentiation: myogenesis is controlled by the cell's shape. *Development* **126**, 3027-3033.

Yang, Y.-P., Li, H.-R., Cao, X.-M., Wang, Q.-X., Qiao, C.-J. and Ya, J. (2013). Second heart field and the development of the outflow tract in human embryonic heart. *Dev. Growth Differ.* **55**, 359-367. doi:10.1111/dgd.12050

Yi, L., Domyan, E. T., Lewandoski, M. and Sun, X. (2009). Fibroblast growth factor 9 signaling inhibits airway smooth muscle differentiation in mouse lung. *Dev. Dyn.* **238**, 123-137. doi:10.1002/dvdy.21831

Yin, C., Kikuchi, K., Hochgreb, T., Poss, K. D. and Stainier, D. Y. (2010). Hand2 regulates extracellular matrix remodeling essential for gut-looping morphogenesis in zebrafish. *Dev. Cell* **18**, 973-984. doi:10.1016/j.devcel.2010.05.009

Zacharias, W. J., Madison, B. B., Kretovich, K. E., Walton, K. D., Richards, N., Udager, A. M., Li, X. and Gumucio, D. L. (2011). Hedgehog signaling controls homeostasis of adult intestinal smooth muscle. *Dev. Biol.* **355**, 152-162. doi:10.1016/j.ydbio.2011.04.025

Zeng, L. and Childs, S. J. (2012). The smooth muscle microRNA miR-145 regulates gut epithelial development via a paracrine mechanism. *Dev. Biol.* **367**, 178-186. doi:10.1016/j.ydbio.2012.05.009

Zeng, L., Carter, A. D. and Childs, S. J. (2009). miR-145 directs intestinal maturation in zebrafish. *Proc. Natl. Acad. Sci. USA* **106**, 17793-17798. doi:10.1073/pnas.0903693106

Zhang, W., Menke, D. B., Jiang, M., Chen, H., Warburton, D., Turcatel, G., Lu, C. H., Xu, W., Luo, Y. and Shi, W. (2013). Spatial-temporal targeting of lung-specific mesenchyme by a Tbx4 enhancer. *BMC Biol.* **11**, 111-111. doi:10.1186/1741-7007-11-111

Zhao, N., Koenig, S. N., Trask, A. J., Lin, C. H., Hans, C. P., Garg, V. and Lilly, B. (2015). MicroRNA miR145 regulates TGFBR2 expression and matrix synthesis in vascular smooth muscle cells. *Circ. Res.* **116**, 23-34. doi:10.1161/CIRCRESAHA.115.303970