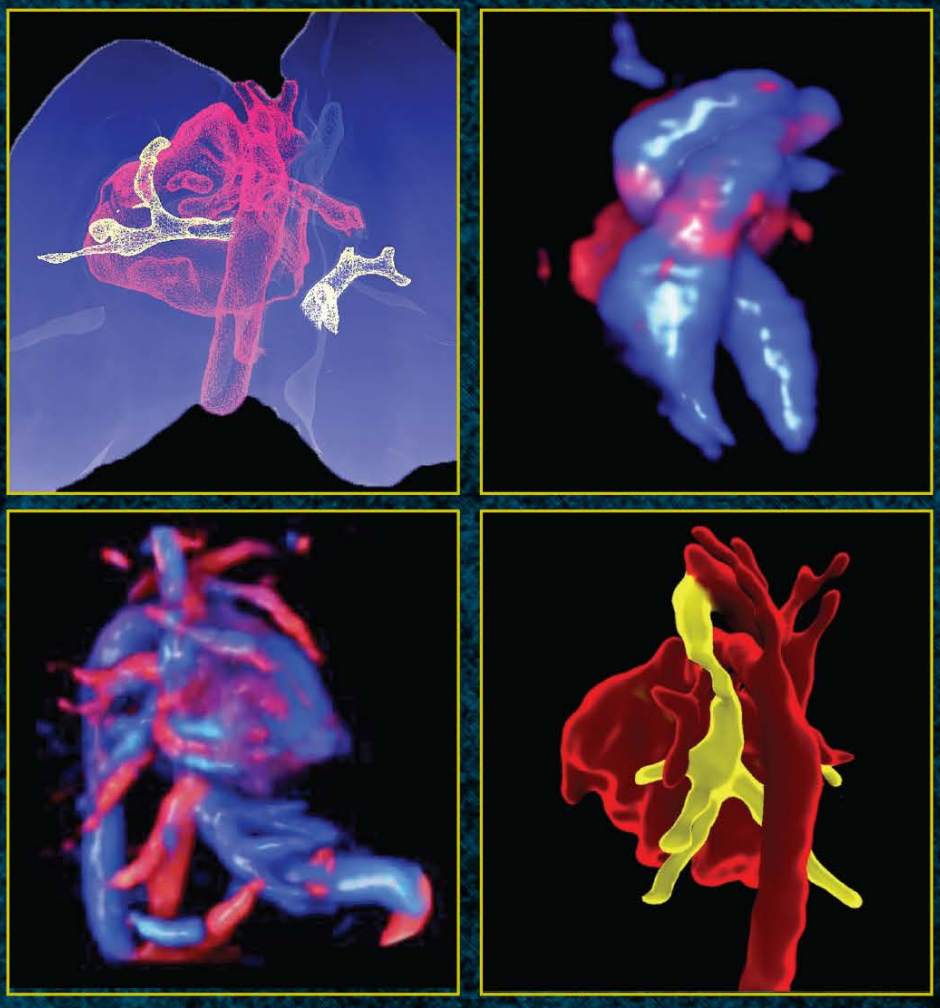
SERIES IN
MATERNAL-FETAL
MEDICINE

Fetal Cardiology

Embryology, Genetics, Physiology, Echocardiographic Evaluation, Diagnosis, and Perinatal Management of Cardiac Diseases

THIRD EDITION



Edited by
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Fetal Cardiology

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CRC Press
Taylor & Francis Group
Boca Raton London New York

CRC Press is an imprint of the
Taylor & Francis Group, an **informa** business

CRC Press
Taylor & Francis Group
6000 Broken Sound Parkway NW, Suite 300
Boca Raton, FL 33487-2742

Printed on acid-free paper

International Standard Book Number-13: 978-1-4987-7176-4 (Pack- Hardback and eBook)

Library of Congress Cataloging-in-Publication Data

Names: Yagel, Simcha, editor. | Silverman, Norman H., editor. | Gembruch, Ulrich, editor.
Title: Fetal cardiology : embryology, genetics, physiology, echocardiographic evaluation, diagnosis, and perinatal management of cardiac diseases / edited by Simcha Yagel, Norman H. Silverman and Ulrich Gembruch.
Other titles: Series in maternal-fetal medicine.
Description: Third edition. | Boca Raton, FL : Taylor & Francis Group, LLC, 2019. | Series: Series in maternal-fetal medicine | Includes bibliographical references and index.
Identifiers: LCCN 2018012336| ISBN 9781498771764 (pack- hardback and ebook : alk. paper) | ISBN 9780429461118 (ebook)
Subjects: | MESH: Heart Diseases--diagnosis | Heart Diseases--therapy | Fetal Heart--physiopathology | Infant, Newborn | Prenatal Diagnosis
Classification: LCC RG618 | NLM WS 290 | DDC 618.3/261--dc23
LC record available at https://lccn.loc.gov/2018012336

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For being our inspiration, for their endless patience, we lovingly dedicate this volume to our wives, Noemie Yagel, Gabi Gembruch, and Heather Silverman

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Preface

This third edition of *Fetal Cardiology: Embryology, Genetics, Physiology, Echocardiographic Evaluation, Diagnosis, and Perinatal Management of Cardiac Diseases* marks a new beginning in our specialty. Like the first two editions, this edition was created through the generosity of the many professionals who shared their expertise: obstetricians, pediatric cardiologists, sonographers, molecular biologists, and medical physicists. This latest edition adds a complement of twelve new chapters, reflecting the immense strides made in recent years. We are delighted to welcome many new contributors, leaders in their specialties writing on diverse topics, to our team.

The fields of fetal imaging and cardiac therapies and interventions are rapidly changing and developing. Whereas in the preface to the second edition we showcased the three-dimensional/four-dimensional revolution in fetal cardiology, the highlight of the third edition is a pair of chapters focusing on fetal cardiac magnetic resonance imaging. This exciting and innovative discipline promises to enhance fetal diagnosis, inform perinatal management and postnatal treatment, and open new avenues in research.

This new edition comprises expanded and revised chapters on treatment options and pharmacological or surgical interventions available to affected fetuses, as well as all life stages of heart disease, from embryology to the neonate, to the reproductive health of women with congenital heart disease and the counseling of families affected by congenital heart disease. Progress in prenatal genetic investigations and counseling is canvassed in a new chapter on chromosome microarray analysis, exome, and whole genome sequencing of the fetal heart. Two chapters are devoted to the complex issue of the intrauterine and postnatal neurodevelopment of fetuses diagnosed with congenital heart disease and the management strategies available to them. An expanded chapter describes the evaluation of fetal cardiac function with advanced Doppler techniques, while another focuses on fetal bradydysrhythmia and the long Q-T syndrome, prior knowledge of which may save lives, not only of the fetus or newborn, but may lead to diagnosis and effective preventative treatment for affected but asymptomatic family members as well.

Congenital heart disease is a broad classification, estimated to affect 8:1,000 live births and to occur at a similar rate in aborti. This underlines the necessity to integrate comprehensive fetal echocardiography in every targeted organ scan. *Fetal Cardiology*, third edition, is a comprehensive guide intended for everyone interested in fetal development: anyone having an interest in the fetal heart, we believe, will find it useful. It is our hope that this volume will bridge the specialties of obstetrics, perinatology, pediatric and general cardiology, and radiology.

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1

Cardiac morphogenesis

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Introduction

Cardiovascular development and the regulatory mechanisms underlying this major embryonic event have become essential knowledge for the fetal cardiologist. The increased potential of ultrasound technology to detect morphology of the growing heart requires more insight into the morphogenetic and epigenetic pathways essential for normal and abnormal development. This area is now expanding with the possibilities of acquiring data from patients by human exome screening, transcriptome analysis, single nuleotide polymorphism (SNIP) technology, and chromatin remodeling.[1–3] It is essential to link these genetic, epigenetic, and environmental clues from patient material to advance our understanding of the complicated interactive processes that govern heart development. The crucial processes in human cardiac development take place within the first 6 weeks of embryogenesis and, as such, cannot be pursued using *in vivo* diagnostics. It is, therefore, still imminent that essential knowledge is incorporated from animal models such as (transgenic) mouse, chicken, and, more recently, zebrafish, as basic principles of heart formation can be compared between various animal models and human development, even profiting from an evolutionary-developmental approach.[4,5] One has to take into account, however, important species differences such as, for instance, a double-sided aortic arch in fish and reptiles, a right-sided aortic arch system in birds, as compared to a left-sided system in mammals,[6] a persisting left-sided caval vein in mice, and the lack of cardiac septation in fish and many reptiles with only a two- or three-chambered heart tube as a final result. The influence of hemodynamics on the developing system has long been underestimated or neglected because of insufficient refined technology to study this *in vivo* in the developing embryo. Currently, newly designed techniques including microparticle image velocimetry have opened up this research field.[7,8] For the fetal cardiologist, particle image velocimetry is a very interesting new development, as noninvasive techniques such as echo-Doppler add physiologic insight to morphology.

The various converging fields of research have sometimes resulted in a confusing use of terminology, which is not easily solved,[9] and which will undoubtedly continue with future new discoveries. This chapter describes in brief the major events in cardiac development.[10] There is a focus specifically on the continuous recruitment of myocardium from the second heart field[11,12] and on extracardiac cellular contributions[13] to the heart and their modulatory role.[14] Genetic and epigenetic causal pathways will be briefly discussed. (For all abbreviations of genes and gene products, see Table 1.1; for all embryological and anatomical abbreviations, see Table 1.2.)

Primary cardiogenesis

The primary heart tube (Figure 1.1a) develops from the precardiac mesoderm, also referred to as the first heart field (FHF) (Figure 1.2), which is located bilaterally in the splanchnic layer of the lateral plate mesoderm of the embryo. These cardiogenic plates fuse rostrally in the midline and form a crescent-shaped structure that will develop into the primary heart tube.[15] The inner lining of this tube is formed by cardiac jelly and endocardial cells that are continuous with the endothelium of the embryonic vascular plexus. The endocardium is most probably a heterogeneous population both in origin and in function depending on the site in the heart. The endoderm of the adjoining developing primary gut plays an important inductive role[16] in the differentiation of the primary heart tube through a cascade of inductive signaling molecules, such as the bone morphogenetic protein (BMP) and fibroblast growth factor (FGF), as well as the inhibitor wingless-related integration site (Wnt) families.[17] The primary heart tube is therefore not just a small homunculus in which all future segments of the heart are already present. This has become generally accepted, and many reviews and book chapters now provide these new insights.[11,14,18,19] Data on the components of the primary heart tube are somewhat confusing, but the most recent data, based on extensive and minute tracing studies, are in favor of an initial small atrial compartment, a myocardial atrioventricular (AV) canal, in which cardiac jelly is remodeled into AV endocardial cushions (putative AV valves),[20] and a primitive ventricle (Figure 1.1b) connecting to the arterial pole.[21] In the human embryo, this primary heart tube already starts to beat with peristaltic contractions at 3 weeks of development. The formed primary heart tube is never completely symmetric (Figure 1.1a), and

Table 1.1 Mentioned genes and gene products

14-3-3 epsilon: Eluted in the 14th fraction on positions 3.3
Actc: Cardiac muscle α actin
Acvr2b: Activin A receptor type B
Alk2: Activing receptor-like kinase
BMP: Bone morphogenetic protein
CHD7: Chromodomain helicase DNA binding protein 7
Cited2: cbp/300-interacting transactivator 2
DSCAM: Down syndrome cell adhesion molecule
eNOS: Endothelial nitric oxide synthase
ET1: Endothelin-1
FGF: Fibroblast growth factor
GATA: Transcription factors binding to the GATA sequence
GJA1: Gap junction α-1 protein
HAND2: Heart and neural crest derivatives
HCN4: Potassium/sodium hyperpolarization-activated cyclic nucleotide-gated channel 4
Isl1: Insulin gene enhancer protein 1
Irx4: Iroquois homeobox protein
KLF2: Krüppel-like factor-2
Lrp2: Low-density lipoprotein-related protein-2
Mef2c: Myocyte-specific enhancer factor
MHC: Myosin heavy chain
MYH 6,7: Myosin heavy chain
NFATc1: Nuclear factor of activated T cells
NKx2.5: Nk2 homeobox 5
Nodal: Member of transforming growth factor superfamily
Notch1: Notch homolog-1
Pax3: Paired box transcription factor
Pitx2c: Paired-like homeodomain transcription factor
Pdgfrα: Platelet-derived growth factor receptor-α
Podoplanin: Encoded by the PDPN gene
Raldh2: Retinaldehyde dehydrogenase-2
RhoA: Ras homolog gene family
SALL4: Spalt-like transcription factor
Shox2: Short stature homeobox
Tbx: T-box proteins
TGFβ: Transforming growth factor
Wnt: Wingless-related integration site
VEGF: Vascular endothelial growth factor

genetic determinants of sidedness[22] and cardiac looping[23] are present. Many mouse knockout studies of genes that are essential for primary cardiogenesis lead to early embryonic lethality. Heterozygous mutations of some of these genes in the human population can lead to congenital malformations such as, for example, those described for Nkx2.5 mutation.[3,24]

Secondary cardiogenesis and organogenesis

Early marker experiments in chicken embryos,[5,16] as well as elegant tracing of cell clones in mouse embryos,[21] have proved that essential parts of the cardiac myocardium at both the outflow and the inflow of the primary heart tube are newly recruited. Transgenic reporter mice with cardiac progenitor-specific marker genes like Isl1, Mef2c, and Nkx2.5 have further supported these findings.[11,18] As the splanchnic mesoderm forming the primary heart tube is referred to as the FHF, the newly recruited cardiac cells derive from mesenchyme referred to as the second heart field (SHF) (Figures 1.1d and 1.2), which is initially positioned medially, but eventually attains a dorsal position between the endoderm of the gut and the primary heart tube. As the dorsal mesocardium is interrupted in its midportion, these SHF-derived cells can only reach the heart tube at the arterial and the venous poles (Figure 1.2). The specific contributions of the SHF to the developing heart are discussed in the next paragraph.

The addition of SHF cardiac cells from a proliferating dorsal pericardial wall source[25] results in a lengthening of the primary heart tube concomitant with ongoing dextral looping that is also governed by genetic factors like asymmetric Pitx2c expression.[23,26]

Recruitment of second heart field cardiac progenitors

Experimental studies show specific characteristics of the addition of cardiac cells. Here details at the outflow tract (OFT) (arterial pole) and inflow tract (venous pole) are described separately.[27]

The arterial pole

Dependent on the marker experiments and reporter gene constructs, several temporospatial patterns of contribution of SHF myocardium have been distinguished, in its most extensive form comprising the complete right ventricle, including the arterial outflow tract and the right side of the ventricular septum[27] (Figures 1.1c and 1.3a). Ongoing scientific insight resulted in changes in nomenclature that might be somewhat confusing (Figure 1.2). The contribution of the SHF-derived cells to the outflow tract is not symmetric, as we and others[28,29] have recently shown. There is a marked deposition toward the (embryonic left) pulmonary side of the OFT forming the right ventricular outflow tract myocardium and components of the pulmonary arterial wall (Figure 1.3b). This process is actually responsible for lifting of the pulmonary orifice anteriorly and to the right of the aortic orifice and as such explains rotation of the orifices and great arteries at the arterial pole (Figure 1.3 and Video 1.1). We have called this anterior SHF-directed process the "pulmonary push."[28] Our

Table 1.2 Embryological and anatomical abbreviations

A	Atrium	MB	Moderator band
Ao	Aorta	MC	Mesenchymal cap
AoS	Aortic sac	MO	Mitral orifice
AP	Arterial pole	NCC	Neural crest cells
AS	Atrial septum	OFT	Outflow tract
ASD	Atrium septum defect	OS	Ostium secundum
AV	Atrioventricular	OTS	Outflow tract septum
AVC	Atrioventricular canal	OVM	Ligament of Marshall
AVSD	Atrioventricular septum defect	PAA	Pharyngeal arch arteries
CAT	Common arterial trunk	PEO	Proepicardial organ
CCS	Cardiac conduction system	PS	Primary interatrial septum
CCV	Common cardiac vein	PT	Pulmonary trunk
CJ	Cardiac jelly	PV	Pulmonary vein
CS	Coronary sinus	RA	Right atrium
CV	Cardinal vein	RC	Right cardinal vein
DA	Ductus arteriosus	RV	(Primitive) right ventricle
DMP	Dorsal mesenchymal protrusion	RVOT	Right ventricular outflow tract
EC	Endocardial cushion	SAN	Sinoatrial node
EPDC	Epicardium derived cells	SB	Septal band
FD	Flow divider	SCV	Superior caval vein
FS	Folding septum	SHF	Second heart field
GCV	Great cardinal vein	SS	Secondary interatrial septum
ICV	Inferior caval vein	SV	Sinus venosus
IFT	Inflow tract	TO	Tricuspid orifice
IS	Inlet septum	TS	Trabecular septum
LA	Left atrium	VCAC	Ventriculo-coronary arterial communication
LCV	Left cardinal vein	VP	Venous pole
LV	(Primitive) left ventricle	VSD	Ventricular septum defect
LVOT	Left ventricular outflow tract		

findings are in line with earlier observations that a specific sensitivity of the pulmonary outflow tract myocardium might relate to distinct genetic coding areas in the subpulmonary and subaortic outflow tract region, which are important for the rotation of the outflow tract.[30]

The venous pole

At the venous pole, the contribution of SHF is important for the growth of the atria. The incorporation of sinus venosus myocardium in the dorsal wall of the atria is an important mechanism (Figure 1.1b). In parallel with the *anterior SHF* at the outflow tract, we have introduced the term *posterior SHF* for this region (Figures 1.1d and 1.2), which is now generally accepted.[11,27,31] We and others have discovered that the sinus venosus myocardium is initially Nkx2.5-negative[32,33] (Figure 1.4a,b).

Tracing of Isl1-positive progenitor cells has shown the extent of the incorporation. In contrast to the outflow tract, this area and the derived sinus venosus myocardium have specific gene expression patterns, including Tbx18,[34] Shox2,[35,36] BMPs,[31,37] and podoplanin.[33] Based on specific gene expression patterns studied in various research centers, there has arisen some controversy concerning whether or not a specific pulmonary venous progenitor myocardium exists.[37–39] A recent publication, using LaacZ tracing of posterior SHF cells, shows the common origin of sinus venosus cells with cells incorporated at the posterior atrial wall, including the pulmonary venous myocardium.[40] Subsequent differentiation with specific gene patterns for each region has most probably led to the controversy.

Not only myocardial wall is added to the venous pole, but also an SHF-derived mesenchymal component, the dorsal mesenchymal protrusion (DMP), is incorporated, being essential for AV septation.[41] The SHF also gives rise to the

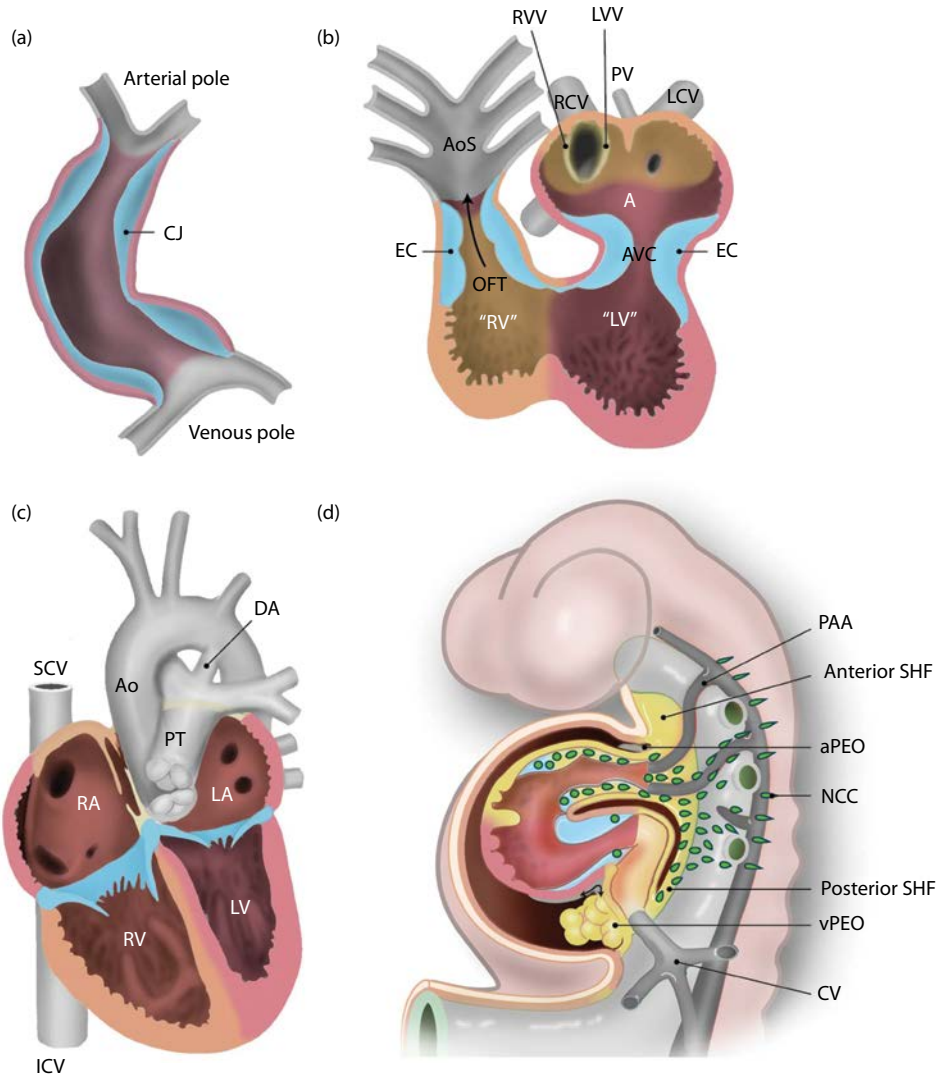

Figure 1.1
Stages of cardiac development. (a) The primary heart tube in an early phase of dextral looping lined on the inside by cardiac jelly (CJ). (b) A more advanced stage of development, depicted for clarity in one plane with the unseptated aortic sac (AoS) and common atrium (A) in a side-to-side position. The entrance of the common pulmonary vein (PV) is on the left side of the atrium is and the entrance of the sinus venosus, flanked by the right (RVV) and left (LVV) valves, on the right side. First (pink) and second heart field (SHF, yellow) derived myocardium is indicated. AV canal (AVC) and outflow tract (OFT) carry the respective endocardial cushions (EC) in blue. The AoS connects to a symmetric set of pharyngeal arch arteries. LCV left cardinal vein, "LV" primitive left ventricle, RC right cardinal vein, "RV" primitive right ventricle. (c) Fully formed four-chambered heart. The interventricular septum is derived from both first and second heart field derived cells. Ao aorta, DA ductus arteriosus, ICV inferior caval vein, LA left atrium, LV left ventricle, PT pulmonary trunk, RA right atrium, RV right ventricle, SCV superior caval vein. (d) Sagittal section of a stage comparable to (b). Migrating neural crest cells (NCCs, green) are depicted, mainly reaching the OFT with few cells to the AV cushions. The proepicardial organ at the venous pole (vPEO) and the smaller one at arterial pole (aPEO) are indicated, although the latter emerges slightly later in development. (CV, common cardinal vein; PAA, pharyngeal arch arteries.) (Modified after Gittenberger de Groot AC et al. In: Moller JH, Hoffmann JIE, eds. *Pediatric Cardiovascular Medicine*, 2nd edn. Wiley-Blackwell; Hoboken, New Jersey, 2012:1–22.[19])

proepicardial organ (Figure 1.1d), which is described in more detail in the section "Epicardium."

The neural crest

A contributing population to the developing heart are the neural crest cells (NCCs) migrating from the crest of the neural tube through the splanchnic mesoderm-derived SHF (Figures 1.1d, 1.2, and 1.5). The relevance of cardiac NCC was first studied in the avian embryo, and its distribution has initially been detected by quail chick-chimera experiments[5,16] and confirmed by retroviral reporter gene transfer[42] showing the deposition of NCC in the arterial outflow tract as well as the differentiation into smooth muscle cells of part of the wall of the great arteries and aortic arch tributaries. At both the

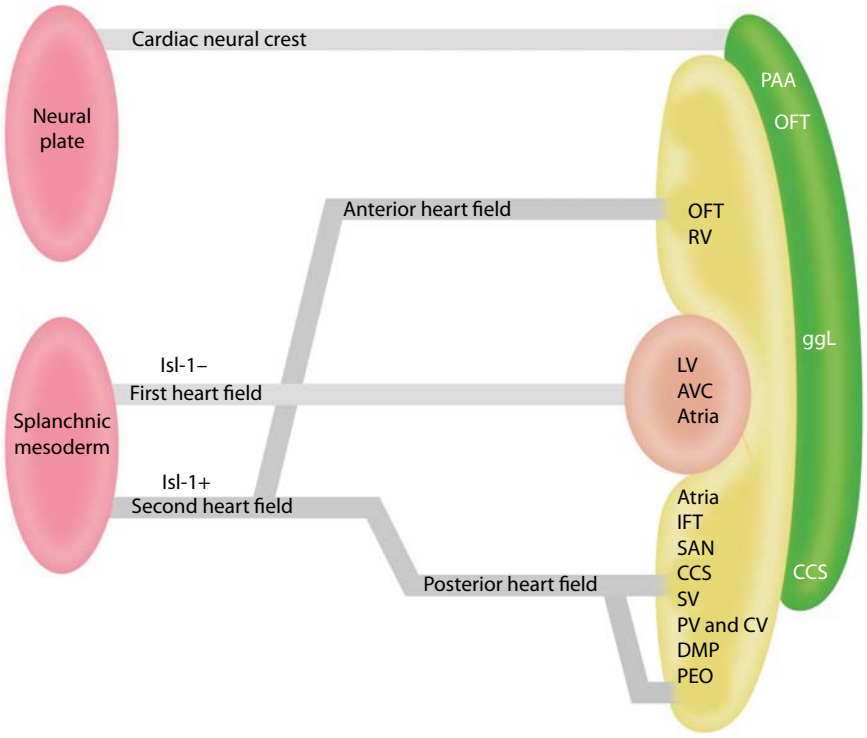

Figure 1.2
The various lineages showing contribution from first and second heart fields as well as the neural crest that arise from the crest of the neural plate and migrate into the pharyngeal arches and the heart. Part of the early splanchnic mesoderm gives rise to the first and second heart field. The first heart field differentiates into the primary heart tube (left ventricle, AV canal and part of the atria). The second heart field separates in an anterior (arterial pole) and a posterior (venous pole) part with many derivatives. (AVC, atrioventricular canal; CCS, cardiac conduction system; CV, cardinal veins; DMP, dorsal mesenchymal protrusion; ggL, autonomic ganglia; IFT, inflow tract; LV, left ventricle; OFT, outflow tract; PAA, pharyngeal arch arteries; PEO, proepicardial organ; PV, pulmonary veins; RV, right ventricle; SAN, sinoatrial node; SV, sinus venosus.) (Modified after Gittenberger-de Groot AC, Poelmann RE. In: Yagel S, Silverman NH, Gembruch U, eds. *Fetal Cardiology*. Informa Healthcare; New York, 2009:9–17.[109])

inflow and outflow tract, NCCs (Figure 1.5a) contribute to the sympathetic and parasympathetic innervation,[43] including a marked ring around the pulmonary venous anlage.[44]

Based on NCC ablation experiments,[42,45] topical deficiency of this cell type was held responsible for many cardiac outflow tract malformations such as common arterial trunk (CAT), pulmonary stenosis and atresia, tetralogy of Fallot, double-outlet right ventricle, and aortic arch malformations. This spectrum is ideally exemplified in the human 22q11 deletion syndrome that also shows other neural crest cell-influenced abnormalities in, for example, the face and thymus. The most essential gene in the 22q11 deletion syndrome, however, is Tbx1[46] that is not expressed in the NCCs but in the SHF mesenchyme providing cells to the arterial pole and, as was recently shown, also to the venous pole.[27] This leads to the important conclusion that it is the disturbed interaction of SHF and NCCs at various levels that is essential for the spectrum of cardiac malformations. It explains also that mutations in a great number of genes expressed in either cell population can evoke comparable malformations, broadening immensely our scope of understanding of the pathomorphogenesis of outflow tract anomalies.

Epicardium

Epicardial cells derive from the posterior SHF and its covering coelomic wall mesothelium (Figure 1.1d). These mesothelial cells not only differentiate into the already described sinus venosus myocardium but also form an epithelial structure at the venous pole next to the sinus venosus referred to as the proepicardial organ (PEO)[47,48] (Figure 1.6a,b). Epicardial cells detach from the PEO and migrate over the initially naked myocardial heart tube.[49] It is evident that retinaldehyde dehydrogenase (RALDH) and retinoic acid play an important role in guiding this process.[50] After covering the heart, the epicardial cells undergo EMT migrating into a mesenchymal subepicardial layer as epicardium-derived cells (EPDCs).[51–53] Subsequently, these EPDCs migrate between the atrial and ventricular cardiomyocytes to form the interstitial fibroblasts and even take up a subendocardial position (Figure 1.6). A second wave of epicardial EMT is seen when the coronary capillary plexus is remodeled into an arterial and venous system in which the EPDCs are the source of smooth muscle cells and periarterial (adventitial) fibroblasts (Figure 1.6c,d). At this stage, the EPDCs are also essential in dissociating the

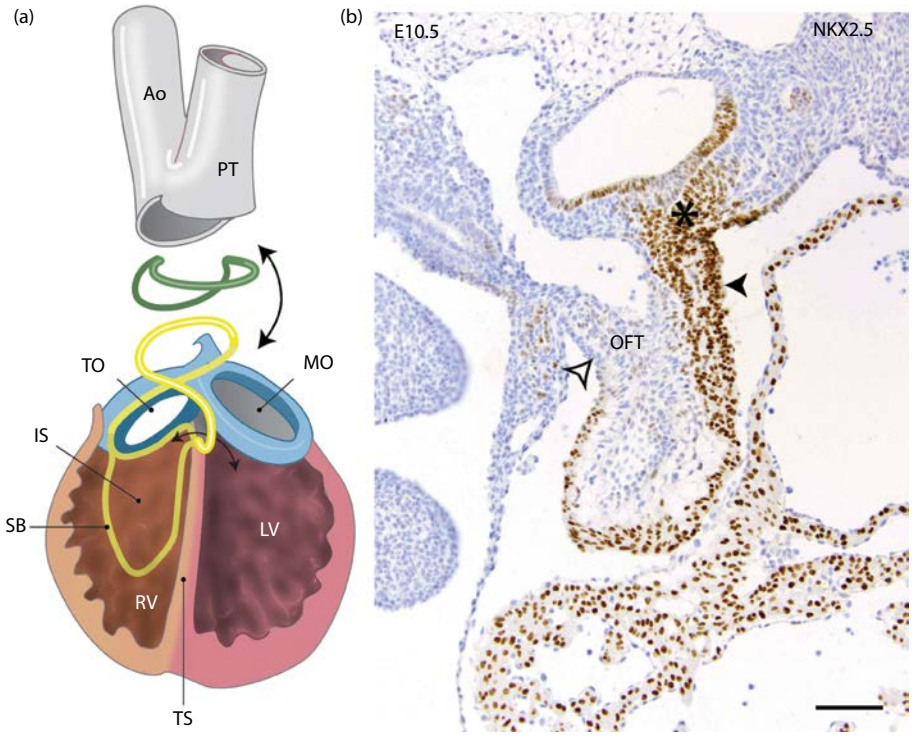

Figure 1.3
(a) Exploded view of the outflow tract with a still unseptated aorta (Ao) and pulmonary trunk (PT). The green ring represents the saddle-shaped semilunar valve level; note that the pulmonary part is more cranial than the aortic part. The curved double-headed arrow represents the pulmonary push. The semilunar valve level is located ventral to the atrioventricular canal (blue) with the mitral (MO) and tricuspid (TO) orifices. The yellow band represents the primary ring, mainly the border between first and second heart field myocardial derivatives. In the right ventricle (RV), the primary ring has expanded to allow formation of the inlet septum (IS) of which the septal band (SB) is the visible representative. The interventricular communication is indicated (small double-headed arrow). (LV, left ventricle; TS, trabecular septum.) (b) Section of the cardiac outflow tract (OFT) of a mouse embryo stained for expression of NKx2.5. The nuclear staining is encountered in differentiated myocardial cells as well as in its second heart field precursors (asterisk) showing a clear asymmetry with a marked preference for the pulmonary side (closed arrow head) as opposed to the aortic side (open arrow head). The pulmonary side is relevant for the pulmonary push.

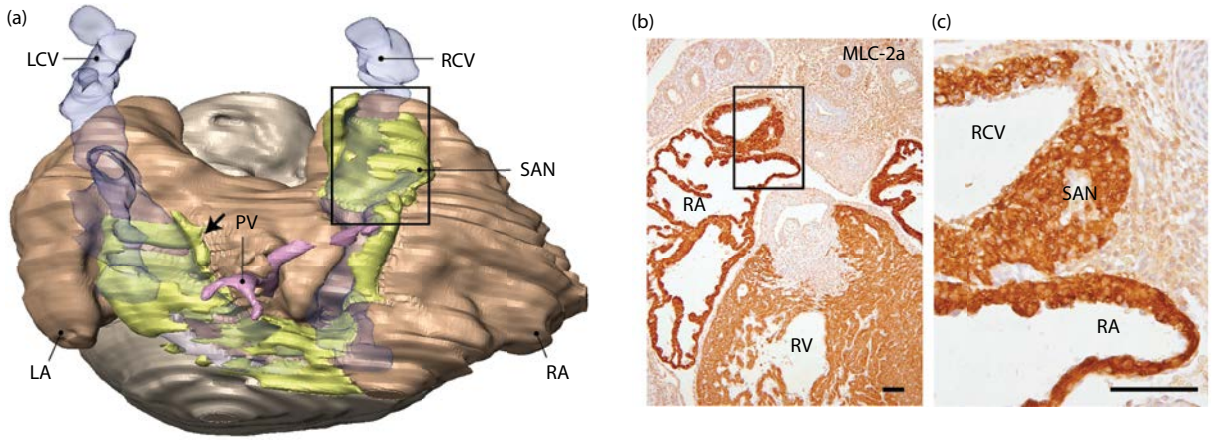

Figure 1.4
(a) Three-dimensional reconstruction of a mouse embryo heart viewed from dorsal. The NKx2.5 negative myocardium (green) is seen as a U-shaped part of the mesoderm connecting and covering the left (LCV) and right (RCV) cardinal veins and encircling the pulmonary vein (PV). At this site, a transient left sinoatrial node (arrow) is seen, while at the right side this is a far larger area that will persist as the definitive right-sided sinoatrial node (SAN). Color codes: right (RA) and left (LA) atria are in brown, the LCV and the RCV in blue, and the PV in pink. (b,c) (magnification) A MLC2a stained section incorporating both Nkx2.5 positive and negative myocardium showing the SAN at the entrance of the RCV into the RA. Note that this segment of the cardinal vein also expresses the atrial myocardial light-chain protein (MLC2a staining) marking it as myocardium. (Modified after Gittenberger-de Groot AC et al. *Anat Rec* 2007;290:115–22.[33])