2

Cardiac Vasculature: Development and Pathology

Michiko Watanabe et al.*
Department of Pediatrics, Division of Pediatric Cardiology, Case Western Reserve University School of Medicine, Rainbow Babies and Children's Hospital, Cleveland OH USA

1. Introduction

Coronary vessels are of particular clinical interest to the general public and adult cardiologists because of their propensity in populations of developed countries to get clogged leading to chest pain (angina) and heart attacks (myocardial infarctions; MIs). Coronary artery disease (CAD) is the leading type of heart disease and the leading cause of deaths in the USA in both men and women (representing 51% of all cardiovascular diseases in 2006; American Heart Association Heart Disease and Stroke Statistics 2010 Update-at-a-glance; http://www.americanheart.org/downloadable/heart/1265665152970DS-3241%20HeartStrokeUpdate_2010.pdf; http://www.nlm.nih.gov/medlineplus/coronaryarterydisease.html). While the developmental biology of cardiac vessels seems removed from this concern for the adult population, findings from this field may be beneficial and relevant for all age groups. Interest in the development of coronary vessels has increased due to findings that suggest the embryonic epicardium provides components of the coronary vessels and factors that positively influence the myocardium. The possibility has been raised that the epicardium might be sufficiently activated to repair cardiac tissue in the adult in part by promoting coronary vascularization. The aim of this Chapter is to call attention to questions regarding coronary vessels that still require answers and to introduce hypotheses that may lead to answers and strategies for cardiotherapy.

2. The structure of coronary vessels and lymphatics in the four-chambered heart (Allen et al., 2007)

The coronary circulation performs critical functions for the heart as in all organ systems in delivering oxygen and nutrients through the arteries and removing deoxygenated blood

* Jamie Wikenheiser[4], Diana Ramirez-Bergeron[3], Saul Flores[1], Amir Dangol[1], Ganga Karunamuni[1], Akshay Thomas[1], Monica Montano[2] and Ravi Ashwath[1]
1 Department of Pediatrics, Division of Pediatric Cardiology, Case Western Reserve University School of Medicine, Rainbow Babies and Children's Hospital, Cleveland OH, USA,
2 Department of Pharmacology, Case Western Reserve University School of Medicine, Cleveland OH, USA,
3 Department of Medicine, Division of Cardiology, Case Western Reserve University School of Medicine, Cleveland OH, USA,
4 Director of Medical Gross Anatomy & Embryology, University of California, Irvine School of Medicine, Department of Anatomy & Neurobiology, Irvine, CA, USA.

and waste through the veins. The lymphatic circulation removes excess fluid within the tissues. In the context of the energy-intensive requirements of the continuously beating heart, the coronary circulation must meet particularly demanding functional requirements that are reflected in its architecture.

The **major vessels** of the heart termed the **coronary arteries** have a very stereotyped architecture (Fig. 1A) that is conserved across individuals within a species and in large part across species (Tomanek et al., 2006b; Sedmera and Watanabe, 2006). With rare exceptions (Frommelt and Frommelt, 2004; Jureidini et al., 1998; Matherne, 2001), the mature four-chambered heart has right and left coronary arteries connected to the aortic lumen by two ostia centrally placed in the right and left sinuses of Valsalva, behind the valvular cusps at the level of the aortic valve. The left main coronary artery (LCA) bifurcates into (1) the circumflex branch of the left coronary artery that wraps around the left atrioventricular groove and (2) the left anterior descending (LAD) coronary artery that courses over the interventricular septum. Other terms for the LAD are anterior interventricular branch of the left coronary artery or anterior descending branch. The right coronary artery (RCA) arises from the right sinus of Valsalva and courses right and posteriorly often with an anterior branch that goes to the sinus node and a posterior branch, the atrioventricular (AV) nodal artery. The main RCA follows the right atrioventricular groove and turns to run along the interventricular sulcus as the posterior descending artery (PDA). In contrast to other tissues that are perfused during systole (end of cardiac contraction when the ventricles are most contracted), the myocardial perfusion of the left ventricle occurs mainly in diastole (when the ventricular lumens are most dilated), while the myocardium of the right ventricle is perfused both during systole and diastole (Epstein et al., 1985; Fulton, 1964; Mosher et al., 1964).

There are rarely variations in the **major coronary arteries**, however there are some variations found in the more distal vessels that have no apparent negative consequences, for example coronary artery dominance. Dominance is determined by what supplies the posterior descending artery (PDA; posterior interventricular artery). In 69% of the population, the right coronary artery is dominant giving rise to the posterior descending coronary artery, which extends to the apex and supplies the posterior part of the ventricular septum, the inferior wall of the left ventricle and the atrioventricular node. In 11% of the population, the left coronary artery is dominant giving rise to the posterior descending coronary artery via the circumflex artery (CF). In 20% of the population it is co-dominant. The dominance has no apparent effect on function under normal circumstances, but is important to note when considering the extent of myocardial damage when a particular artery is occluded or negatively affected. The major coronary arterial system distributes blood to the microcirculation such as capillaries and postcapillary venules which are the main sites of interchange of gas and metabolite molecules between the tissue and blood.

The **major cardiac veins** run along similar avenues as the major arteries. The middle cardiac vein runs within the epicardium from the apex to the base on the posterior surface of the interventricular groove and connects to the coronary sinus. This vein is generally paired with the PDA. Anterior cardiac veins connect to the small cardiac vein that runs posteriorly along the right atrioventricular groove with the right coronary artery and connects to the coronary sinus where the middle cardiac vein also connects. The great cardiac vein courses along the anterior interventricular groove from the apex to the base and wraps around the left atrioventricular groove posteriorly and connects to the coronary sinus. The coronary sinus ultimately drains into the right atrium (Gensini et al., 1965; Gilard et al., 1998).

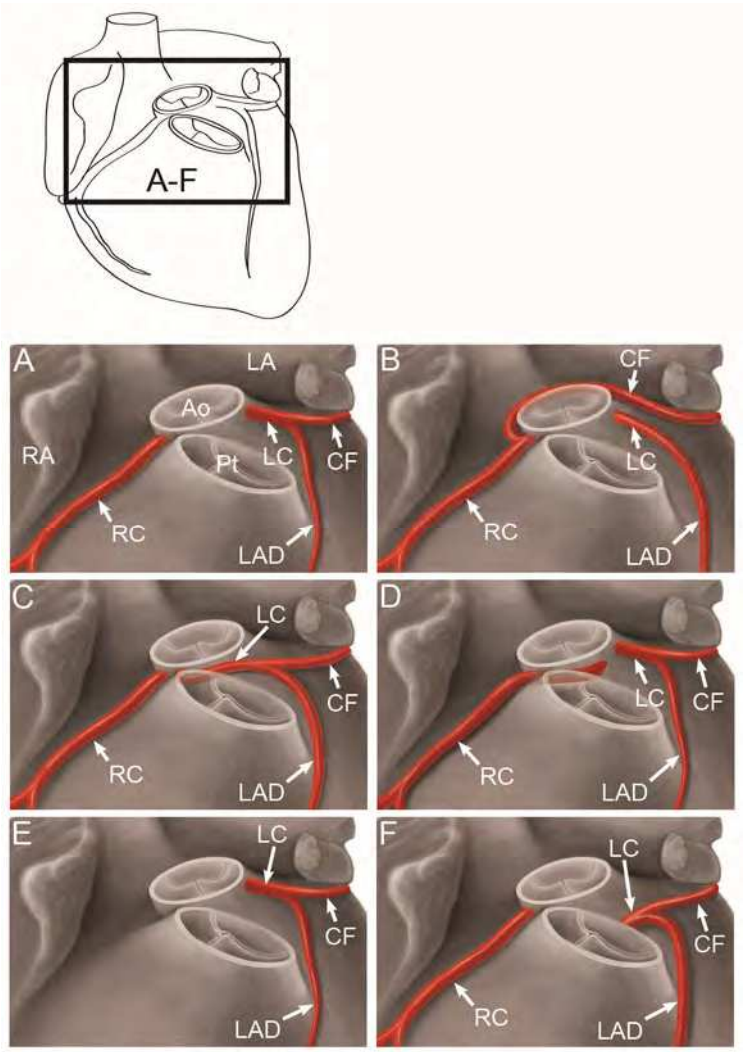


Fig. 1. **Normal morphology and the most frequently seen congenital coronary anomalies.** **A**: Normal coronary artery morphology. **B**: The circumflex artery originating from the right coronary artery near the right sinus of Valsalva. This is the most common coronary artery anomaly (35% of cases). **C**: The left coronary artery originating from the right sinus of Valsalva. This anomaly carries an increased risk of sudden death. **D**: The right coronary artery originating from the left sinus of Valsalva. The frequency of this anomaly is around 30%. **E**: A single coronary artery originating from either the left or right sinus of Valsalva. The frequency of this anomaly is between 5-20%. Note only a single left coronary is shown. **F**: The left coronary artery originating from the pulmonary artery. LA, left atrium; RA, right atrium; Ao, aorta; Pt, pulmonary trunk; RC, right coronary; LC, left coronary; LAD, left anterior descending; CF, circumflex. (Illustrations by Laura M. Bock, BFA).

The cardiac **lymphatic system** (Miller, 1982) also has its larger vessels within the epicardium but their anatomy is not often covered in commonly used textbooks. Surgeons concern themselves with the connection of the largest lymphatics such as the thoracic duct that drains most of the body except the right upper quadrant and usually connects at the junction between the left internal jugular and left subclavian veins. These are not strictly cardiac lymphatics as they are outside the heart. The largest distributing lymphatic vessels within the heart are found in the epicardium running alongside the larger blood vessels. These are connected to a meshwork of lymphatic capillaries that lie within the myocardium mainly in the ventricular walls. While the epicardial lymphatics are documented in several studies, the findings regarding the myocardial lymphatics of the adult heart are controversial and complex and will be discussed separately in a review in preparation (Thomas, A., Watanabe, M. et al., personal communication).

Thus the largest cardiac vessels whether arteries, veins or lymphatics are found embedded within the thick epicardium of the sulcus regions as in the atrioventricular groove and the dorsal and ventral interventricular grooves. This pattern raises several questions. How is this stereotyped pattern of the major named coronaries and veins set up and maintained? Why do the largest coronaries course within the epicardium and do not end up more often within the myocardium as in the cases of myocardial bridging? Why do the left and right coronary arteries normally connect only in two places at the left and right cusps of the aorta and not at the other posterior aortic cusp or at the cusps of the pulmonary artery? What regulates the density of vessels within the epicardium and myocardium and their diameters? The answer to these questions may aid us on intervening in cardiac disease by coaxing collateral growth or enhancing vascular density when myocardial infarctions are a danger or prior to major cardiac surgery. While these fundamental questions have yet to be definitively answered, some hypotheses emerge from studies of the events during coronary vessel development in the embryo and fetus that will be discussed in subsequent sections.

3. Coronary anomalies and consequences. Clinically significant anomalies associated with sudden death and propensity to ischemic heart disease

The major coronary arteries generally follow a specific morphological template (Fig. 1A). Variations from this morphology are rare and may not have clinically significant consequences but are nonetheless important to know about prior to procedures in cardiac surgeries or when inserting leads for pacing for electrophysiological studies. However, specific classes of anomalies have been associated with an increased risk of sudden and exercise related death (Cheitlin and MacGregor, 2009; Eckart et al., 2006a; Frescura, 1999; Haugen and Ellingsen, 2007; Taylor et al., 1992).

Overall, coronary artery anomalies represent a rare and small group of malformations, that are nonetheless important. These anomalies may be isolated or occur as a part of complex congenital heart diseases or associated with hypertrophic cardiomyopathy, dilated cardiomyopathy and sudden cardiac death.

The clinically significant anomalies are near the proximal attachment to the aorta and have been associated with exercise-related deaths. The anomaly in which the left circumflex arises from the right main coronary artery passing posterior to the aorta is the most common coronary anomaly accounting for about 35% of coronary anomaly cases (Fig.1B). There are usually no clinical complications, but compression by the aorta and mitral valve has been reported. In the latter case, implantation of a prosthetic fixation ring could be considered.

This implantation is a series of procedures for the replacement of the mitral valve with an artificial mitral valve and supporting ring (Chiam and Ruiz, 2011).

Another anomaly in which the **left coronary artery arises from the right sinus of Valsalva (ARCA)** and passes between the aorta and the pulmonary artery is rare (3% of cases of coronary anomalies), but has been associated with sudden death (Fig.1C). In this case, it is presumed that during exercise, the abnormal position of the coronary artery running between the aorta and pulmonary trunk causes it to be compressed by the dilated arteries during exercise thus reducing blood flow to a large portion of the heart. There are 4 possible routes for the left main coronary artery (1) posterior to the aorta, (2) anterior to the right ventricular outflow tract, (3) within the ventricular septum under the right ventricular infundibulum, and (4) between the aorta and the right ventricular outflow tract (Fig.1C). Because this last variant carries an increased risk of sudden death, surgical reimplantation may be necessary.

Taylor et al. (Taylor et al., 1992) reviewed the records of 242 patients with isolated coronary artery anomalies and determined that sudden death and exercise related death were most common when the origin of the left main coronary artery came from the right coronary sinus. High risk anatomy involved abnormalities of the initial coronary artery segment or coursing of the anomalous artery between the aorta and pulmonary artery. Frescura et al. (Frescura et al., 1998) analyzed the anatomic collection of 1,200 specimens of individuals with congenital heart disease and anomalous origin of coronary arteries was observed in 27 of these individuals (2.2%). This study concluded that more than half of the postmortem cases with an anomalous origin of the coronary arteries died suddenly. Eckart et al., (Eckart et al., 2006b) reviewed the autopsy reports of sudden cardiac deaths involving U.S. military recruits during basic training from 1977 through 2001. This study found that all sudden cardiac deaths resulting from anomalous coronary origin involved a left main coronary artery originating from the right coronary sinus with a course between the aorta and the right ventricular outflow tract and an otherwise normal distribution of the other major epicardial coronary arteries.

Anomalous origin of right coronary arterial branches from the left sinus of Valsalva occurs in approximately 30% of all major coronary arterial anomalies (Fig.1D). In this condition, the right coronary artery runs between the aorta and the right ventricular outflow tract. Since it carries increased risk for sudden death as does certain cases of ARCA, surgical reimplantation is recommended.

The **anomalous left coronary from the pulmonary artery (ALCAPA;** Bland-White-Garland syndrome; Fig. 1F) results in left ventricular insufficiency or infarction and infants with this condition have a high mortality rate; 65-90% die before age 1 from congestive heart failure (Park, 1988; Pena et al., 2009). This anomaly is usually isolated but can be associated with other congenital heart anomalies such as Tetralogy of Fallot or coarctation of the aorta. It usually presents at birth or shortly after with an increase in myocardial ischemia followed by exhaustion of the coronary vascular reserve. Due to the transition in the neonatal circulation, the pressure in the pulmonary artery remains high. As the pulmonary artery pressure drops, there is more flow from the aorta into the pulmonary artery through the coronary circulation. This results in decreased perfusion of the myocardium and leads to myocardial ischemia. Initially this condition may be transient but with increased exertion, it progresses to infarction of the antero-lateral left ventricle or free wall with dysfunction. Mitral regurgitation can develop secondary to left ventricular delectation and infarction and/or dysfunction of the anterolateral papillary muscle. Diffuse endocardial fibroelastosis of the left ventricle and thickening of the anterior mitral valve leaflet can also occur.

Infants may present with heart failure and the signs of myocardial infarction in the form of irritability with feeding or activity. Older children may be asymptomatic or may have dyspnea, syncope, or angina. Sudden cardiac death after exertion has been known to occur (George and Knowlan, 1959).

The electrocardiogram shows abnormal Q waves in the leads I, aVL and pre-cordial leads V4 to V6. Noninvasive imaging like echocardiography will confirm the diagnosis in most of the cases. If uncertain, angiography or computed tomography (CT) scan with higher resolutions can complement and confirm the diagnosis and also provide insight into the extent of collaterals and help in sorting out which coronary artery is dominant.

About 87% of the patients present in infancy (Neufeld, 1983) and of these 65% to 85% die before one year of age due to congestive heart failure and infarction (Wesselhoeft et al., 1968). It is noted that if the children improve spontaneously (Liebman et al., 1963), it might be from formation of extensive collaterals or there could be ostial stenosis at the entry of the anomalous coronary into the pulmonary artery, thus conferring slight protection. However, they are still at high risk of sudden cardiac death with exercise (Fontana, 1962).

The treatment of such a condition is surgical implantation of the left coronary artery into the aorta (Grace et al., 1977; Huddleston et al., 2001; Jin et al., 1994; Schwartz et al., 1997). An alternative is the Takeauchi procedure, in which an aorto-pulmonary window is created with a tunnel that leads the blood from the aorta to the coronary artery (Takeuchi et al., 1979).

ALCAPA has been separated into the infant type and the adult type (Pena et al., 2009). The infant type is as described earlier in this paragraph. The rare adult type is hypothesized to be the cause of sudden cardiac death that occurs in 80-90% of these individuals. Survival of the adult with ALCAPA is possible because of the development of collaterals between the left and right coronary arteries. In these cases there is likely a pulmonary-coronary steal with a left to right shunt. These account for 15% of ALCAPA patients where the myocardial blood flow can sustain myocardial function at rest or even during exercise allowing these individuals to reach adulthood (Neufeld, 1983).

Even with these collaterals, there is not enough circulation to the left ventricle resulting in ischemia. Surgical repair is usually required and is carried out for the infant by direct reimplantation of the origin of the left coronary artery into the aorta with a "button" (small segment) of pulmonary artery.

Single coronary artery (Fig. 1E): At a frequency of 5-20% of coronary anomalies, a single coronary artery arises from the aorta and then branches to give rise to the right and left coronaries (Ogden, 1970; Shirani and Roberts, 1993). As many as 40% of single coronary artery cases are associated with other congenital cardiac defects such as Tetralogy of Fallot. This anomaly carries a mildly increased risk of sudden death. The branches can pass between the two great arteries, resulting in compression. Of course with only one trunk there is increased vulnerability. For example, atheroma formation (swelling and accumulation of material) at the single trunk can be critical. Surgical repair is considered on a patient to patient basis.

Coronary artery fistulae [reviewed in (Luo et al., 2006; Schamroth, 2009) and (http://emedicine.medscape.com/article/895749-overview)]: Coronary artery fistulae (CAF) are major abnormal connections between a coronary artery and a cardiac chamber (coronary-cameral fistula) or inappropriate vessels and vascular structures (coronary arteriovenous fistula for coronary arterial-venous fistulae; CAVFs) and occur in 50% of coronary vasculature anomalies. For the most part, fistulas are small, have no untoward consequences to hemodynamics, and require no clinical intervention. These are only discovered when echocaradiography or coronary arteriography are performed for other reasons. Larger fistulae may continue to enlarge and eventually cause the "coronary artery steal phenomenon" in

which blood flow to the myocardium is compromised and may cause ischemia during increased activity. These fistula require surgical or percutaneous intervention for their closure. It is proposed that these inappropriate connections are remnants of the primitive coronary network that have failed to remodel and regress appropriately.

The range of **symptoms** associated with coronary artery anomalies can vary based on the specific type of lesion. However, the general concept is based on an imbalance between supply and demand of myocardial perfusion and also due to the steal phenomenon associated with left to right shunting. There are three coronary anomalies associated with myocardial ischemia, infarction, and/or lethal arrhythmias which could then lead to fibrillation or electromechanical dissociation. These are ALCAPA, coronary artery arising from the wrong sinus and coursing between the two great arteries, and large coronary artery fistula.

A history of unexplained syncope (loss of consciousness or fainting) or syncope associated with exercise should raise a diagnostic possibility of an anomalous coronary artery. Initial tests would include an electrocardiogram to rule out arrhythmia, left ventricular hypertrophy or prior evidence of ischemia. An echocardiogram is very helpful in identifying the coronary artery origin and its proximal course should be studied with utmost care. Hypertrophic cardiomyopathy should be ruled out using this method. If the echocardiogram raises the suspicion and is unable to provide enough information, a transesophageal echocardiogram, magnetic resonance imaging and/ or CT scan may be more sensitive and should be considered (Schmitt et al., 2005).

While the association between sudden death and coronary anomalies has been made for certain such anomalies, no treatment has been established for individuals identified with these anomalies but suffering no symptoms.

Classification of coronary artery anomalies: Coronary artery anomalies can be classified as abnormal origin of the coronary arteries from the wrong aortic sinus, anomalous origin of the left or the right coronary artery from the pulmonary artery, absence of a coronary artery, and congenital coronary artery fistulas (See Table 1).

Significant congenital anomalies of the coronary arteries
I. Without congenital heart disease
A. Coronary arteries arising from the wrong sinus
i. without intramural course
ii. with intramural course (within the media of the great vessels)
B. Anomalous origin of the coronary arteries from the pulmonary arteries
i. ALCAPA*
ii. ARCAPA.*
C. Single coronary artery or absence of a coronary artery
D. Coronary artery fistulas
E. Myocardial bridging
II. Coronary artery anomalies associated with congenital heart disease
A. Transposition of the great arteries
B. Tetralogy of Fallot
C. Pulmonary atresia with intact ventricular septum
D. Aortic atresia with mitral atresia/stenosis

*ALCAPA: anomalous origin of the left coronary artery from the pulmonary artery;
*ARCAPA: anomalous origin of the right coronary artery from the pulmonary artery.

Table 1.

Myocardial bridges are another class of coronary anomalies where an artery that normally confines its path within the epicardium dives and travels several millimeters within the myocardium before the terminal branches [Reviewed in (Boktor et al., 2009; Hakeem et al., 2010; Sunnassee et al., 2011; Vales et al., 2010)]. Myocardial bridging has been described as early as 1737 (Rayman, 1737) and have been described since with a wide range of frequencies reported. In one study of 200 adult human hearts collected from autopsies, myocardial bridges were found in 34.5% with a mean length of 31 mm and a mean depth of 12mm (Loukas et al., 2006). The most common site of myocardial bridging was over portions of the left anterior descending coronary artery (LAD). Myocardial bridging usually does not carry a significant increase in risk of sudden death, however ischemia has been reported, in particular when associated with hypertrophic cardiomyopathy. Angiography has identified compression of arteries at the site of myocardial bridging during systolic contraction (Laifer and Weiner, 1991). Surgical repair is considered in select cases to remove the myocardial bridge when there are symptoms such as angina or myocardial infarction. Many patients with myocardial bridges are asymptomatic, but these bridges are hypothesized to lead to a tendency to develop myocardial infarction (MI), ischemia, and other cardiac problems. Long bridges are associated with negative cardiac symptoms (Bourassa et al., 2003). Paradoxically myocardial bridges have been proposed to lead to a decrease or an increase of atherosclerosis. Myocardial bridges correlate with left coronary artery dominance and are thought to be the result of developmental events. Why myocardial bridges and coronary artery dominance would be related is a mystery.

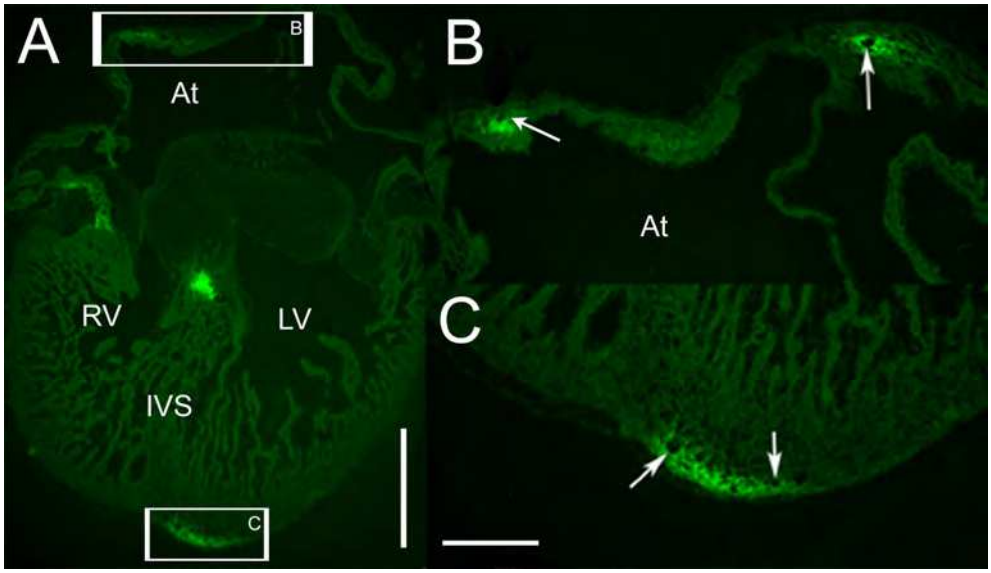


Fig. 2. EF5 at hypoxic regions where major coronary vessels will differentiate. A: EF5 staining in frontal sections of st 30 chicken hearts B: Higher magnification of a region similar to that in the box in A. C: Higher magnification of a region indicated by the box in A. Arrows indicate lumens of small vessels. Scale bar in A = 500 μm and 200 μm in B & C. (Wikenheiser et al., 2006).

Summary: The coronary artery anomalies discussed above are presumed to arise in the embryonic or fetal stages of cardiovascular development from as yet unknown causes. Recent investigations described in Section V, suggest that some coronary anomalies may involve disturbance of a "hypoxic template". Anomalies in the proximal arteries resembling those observed in clinical specimens have been reproduced in avian embryos subjected to conditions that would alter this hypoxic template (Fig 2). ;(Wikenheiser et al., 2009).

Much less attention has been paid to anomalies of the cardiac veins and lymphatics. In one case study, the importance of venography prior to inserting a pacing lead into the coronary sinus was underscored because the contrast agent indicated that the lead may have entered an anomaly of the middle cardiac vein (Yuniadi et al., 2004).

4. Development of coronary vessels

The proepicardial serosa and the embryonic epicardium are known to be sources for the components of the cardiac vessels in the embryo. Recent evidence suggests that the adult epicardium can also be activated to contribute to the vasculature and even the cardiomyocyte population. A number of apparently conflicting interpretations of the data and caveats have been raised that are worth discussing regarding this important cardiac tissue.

The proepicardium and the embryonic epicardium have been the focus of and continue to be the focus of much study [reviewed in (Gittenberger-de Groot et al., 2010; Olivey et al., 2004; Ratajska et al., 2008; Tomanek, 2005; Wessels and Perez-Pomares, 2004)] because it is the source of many cell types critical for the heart including components of the vasculature. The embryonic epicardium arises from the serosal covering of the body wall between the sinus venosus and the future liver. The mesothelial cells at this caudal border of the pericardial cavity form villi and become a small mass appearing as a wrinkled "cauliflower-shaped" structure that is called the proepicardium (PE), proepicardial serosa, or proepicardial organ (PEO) (Manner, 1993). This structure grows up against the atrioventricular groove and migrates to the heart apparently assisted by extracellular matrix fibers that bridge the gap between the myocardium and the proepicardium (Nahirney et al., 2003; Olivey et al., 2004). This structure extends many tissue processes and covers the surface of the naked myocardium of the looped heart in avian species (Hiruma and Hirakow, 1989; Ho and Shimada, 1978; Viragh and Challice, 1981). Bone Morphogenetic Protein (BMP) has been identified as a factor that may be involved in controlling the timing and direction of proepicardial protrusion towards the heart (Ishii et al., 2010). The transcription factor GATA4 has been found to be strongly expressed in the proepicardium and is required for the formation of the epicardium (Watt et al., 2004). When the GATA cofactor FOG-2 is absent in mice, the coronary vasculature does not develop (Tevosian et al., 2000; Tomanek, 2005). The molecular and cellular signals that initiate the growth and coverage of the cardiomyocytes of the embryonic heart are not known. It is likely that proepicardial growth and epicardium coverage would be coordinated closely with the level of hypoxia in cardiac tissues and the contractile function of the heart.

The transition from proepicardium to epicardium is thought to differ between species (Nesbitt et al., 2006). While avians and rats undergo the process as described above, mice and zebrafish may do it a different way. In the mouse, the proepicardial processes appeared

to be released as vesicles from the proepicardial serosa that float in the pericardial space and attach to the surface of the myocardium (Viragh and Challice, 1981). Whatever method is used for a particular species, the signal that induces the growth and attachment of the proepicardial processes or vesicles is incompletely understood but likely involves signals from the thickening myocardium and perhaps the endocardium.

Immediately after proepicardial processes or vesicles attach to the surface of the myocardium, an extracellular matrix accumulates between the single layer of mesothelial cells and the myocardium. The epicardial coverage occurs in a stereotyped pattern in the avian heart with the dorsal surface of the atrioventricular junction covered first and radiating out from there, wrapping around the atrioventicular groove, the ventricles and atria and finally the distal portion of the outflow tract (Hiruma and Hirakow, 1989; Ho and Shimada, 1978). This distal portion of the outflow tract has been shown to be covered by epicardial cells from another more cephalic source even when the proepicardial serosa is ablated or impeded (Gittenberger-de Groot et al., 2000). These cells have a different morphology (more cuboidal than squamous), different gene expression, and may also have different capabilities and properties (Perez-Pomares et al., 2003).

The embryonic epicardium consists of a single layer of mesothelial cells, underlying connective tissue, and mesenchymal cells within the connective tissue. These mesenchymal cells arise from epithelial-mesenchymal transition (EMT) of the mesothelium but may also come from the sinus venosus or liver primordia (Perez-Pomares et al., 1997; 1998; Dettman et al., 1998). The cells that undergo EMT are termed epicardial derived cells (EPDCs) and have been tracked into the subepicardium, myocardium and even into the endocardium at sites where valves form (Gittenberger de Groot et al., 1998). Some EPDCs differentiate into the components of the coronary vasculature.

Epicardial EMT is stimulated by vascular endothelial growth factor (VEGF), fibroblast growth factors (FGF-1, FGF-2 and FGF-7) and epidermal growth factor (EGF), but is inhibited by transforming growth factor (TGFb-1-3) (Morabito et al., 2001). The proposed inhibitory role of TGF-β in epicardial EMT is in contrast to its positive role in endocardial EMT and is still seen as controversial (Compton et al., 2006; Olivey et al., 2006). When the myocardium in the embryonic mouse produces an excess of angiopoietin-1 (Ang1), the epicardium fails to develop and that results in an absence of coronary vessels and death (Ward et al., 2004).

The epicardium as a source for cardiac cell types. Lineage studies were conducted using retroviral labeling by injection of engineered virus into the proepicardium (Mikawa and Fischman, 1992). As retroviruses were used for the transfection of reporter genes, the genes integrate into the genome and clonal analysis is possible over many stages of development. In the case of these studies, the reporter gene was the bacterial *lac-z* gene expressing the enzyme beta-galactosidase that can be detected using a dye substrate that turns into a blue precipitate within the cell expressing the reporter gene. The analysis of individual discrete clones of blue cells revealed that there were clones with only endothelial cells or clones with smooth muscle cells and fibroblasts, but no clones were found that included the combination of endothelial cells and smooth muscle cells or endothelial cells and fibroblasts. The findings suggested that there are two populations of precursor cells in the proepicardium, one population that became endothelial cells and a separate population that became smooth muscle cells and fibroblasts. In this study, the virally marked proepicardial cells could have been either the mesothelial cells or the mesenchymal cells of the proepicardium.

Many studies using different approaches confirm that the epicardium can give rise to vascular smooth muscle cells and fibroblasts. However, some lineage tracing approaches do not clearly support that the epicardium serves as a source of endothelial cells. Furthermore, the potential for the epicardium to provide precursors of cardiomyocytes is an exciting idea for cardiac therapy, but has been even more controversial and will be discussed below.

4.1 Where do endothelial cells in the heart come from?

The origin of endothelial cells is still somewhat controversial with several theories being discussed. The theories can be separated into three.

1. The epicardial mesothelial layer undergoes epithelial-mesenchymal transition (EMT) and gives rise to EPDCs (epicardial derived cells) that travel into the epicardium or myocardium and become endothelial cells that form vascular tubes and vessels by vasculogenesis.
2. Endothelial precursors travel within the connective tissue of the epicardium as mesenchymal cells, but do not derive from the epicardial mesothelium. These precursors use the connective tissue of the epicardium as a conduit as do neural crest cells and may originate from outside the heart in the liver primordia.
3. Precursors come from the sinus venosus and veins that transdifferentiate into endothelial cells of the arteries and use the epicardium as a conduit as in (2) (Red-Horse et al., 2010).
4. Endocardial cells differentiate into endothelial cells of the myocardium.

Our findings suggest that lymphatic endothelial precursor cells may travel from outside the heart using the epicardial lining of the outflow tract to travel into the heart (Karunamuni et al., 2010) supporting scheme (2). We also have evidence that lymphatic endothelial cells may also come from precursors within the epicardium.

The Cre-Lox method of lineage tracing has been used widely to trace cell fate and to find evidence for coronary vessel precursors so it is worth discussing the caveats of using this technique. Findings using this method have suggested that cardiomyocytes and few if any endothelial cells arise from the embryonic epicardium. Concerns have been raised regarding the lineage tracing of epicardial cells by this method. The Cre-mice available include, Wt1-Cre, TBX18-Cre and Gata5-Cre, that do appear to allow epicardial specific expression when analyzing limited areas of the heart at certain stages (Cai et al., 2008; Sridurongrit et al., 2008; Zhou et al., 2008). However, these may not be as specific as desired. For example, Wt1-Cre is expressed by cardiac progenitors in the secondary heart field so it may label the lineage prior to their split into myocardial and epicardial lineages. TBX18 is known to be expressed in a subset of cardiomyocytes that are not of epicardial origin (Christoffels 2009). Cre expression can be irreversibly turned on even if the expression of the gene is expressed transiently and in low levels. This expression would be difficult to detect and characterize histologically by immunostaining or in situ hybridization for TBX18 itself.

To address this problem in studies of the role of the epicardium in the adult mouse heart, the Wt1-CreERT2 tamoxifen-inducible reporter constructs have been used (Smart et al., 2011; Zhou et al., 2011). This strategy allowed the investigators to label with a fluorescent reporter (YFP) only those cells expressing Wt1 at the time of tamoxifen injection. Wt1 and YFP expression appeared to be epicardial specific in the adult. However, the same criticism

could be invoked for this strategy as for the strategy used to mark the epicardium in the embryo. Thus, transplantation of lineage labeled and explanted epicardial cells was used to support the findings using the Cre-line (Zhou et al., 2011).

Given the uncertainties of the Cre-Lox method of lineage-tracing, several groups have found that the epicardial/mesothelial Cre lineage tracing (Wt1-Cre, TBX18-Cre) rarely if ever provided endothelial cells to the heart or other organs (Cai et al., 2008; Que et al., 2008; Wilm, 2005; Zhou et al., 2008). Other groups including ours found that endothelial cells are indeed part of the Wt1-Cre lineage (personal communication). The staining protocol, background of the mice, and the Cre-construct might be the variables that result in these differences in findings between laboratories.

In summary, multiple avenues for endothelial cells precursors to contribute to the heart have been proposed and at this point, all may be correct.

Coronary vascular development. Several reviews summarize what is known about coronary vascular development (Lie-Venema et al., 2007; Olivey et al., 2004; Olivey and Svensson, 2010; Reese et al., 2002; Tomanek, 2005; Wada, 2003; Wessels and Perez-Pomares, 2004). Precursors of the coronary vasculature are contributed by the embryonic epicardium that arises from the pro-epicardial serosa at a stage when chamber differentiation has begun (Hiruma and Hirakow, 1989; Ho and Shimada, 1978; Viragh and Challice, 1981). The function of the primitive coronary vascular system prior to exhibiting the mature pattern of two arterial connections with the aortic lumen is not known. Are they already moving blood and fluid within the heart? What are the hematopoietic properties of epicardial cells?

Quail endothelial precursors and hemangioblasts are labeled by an antibody Qh-1 (Eralp et al., 2005; Kattan et al., 2004) and appear as individual cells along the back (dorsal surface) of the atrioventricular junction (AVJ), eventually covering the entire heart (Fig. 5). These cells accumulate around the proximal OFT and right ventricular base to form a peritruncal ring of anastomosing capillary-like vessels that surrounds the proximal OFT myocardium and cranial portion of the ventricles in the epicardium (Fig.6). Some vessel precursors enter the myocardium and make multiple connections to the aortic lumen. Subsequent remodeling results in maturation and maintenance of the roots of the right and left coronary arteries connecting to the mature branching vasculature (Tomanek et al., 2006a; Tomanek et al., 2006b; Waldo et al., 1990). Early assembly of coronary vessels occurs by vasculogenesis, the formation of blind-ended tubes that connect with each other to form continuous vessels (Kattan et al., 2004; Mikawa and Fischman, 1992). Once these tubes are connected to each other and to the aortic lumen, angiogenesis, the growth of vessels from preexisting vessels, becomes a prominent mechanism for coronary growth. The signals that promote the formation of the nascent tubes and their eventual connection to each other and to the aortic lumen are incompletely understood**.** Another equally important issue is the remodeling that eliminates nascent vessels during the transformation of the primitive vascular network into a mature branching vasculature.

To complicate this picture of coronary vessel development, Qh-1 labels not just blood vessel precursors but distinctly labels lymphatic precursors (Parsons-Wingerter et al., 2006) in the chorioallantoic membrane (extraembryonic tissue) and we have recently found that the same is true in the developing epicardium (Karunamuni et al., 2010). Therefore, some of the structures labeled with Qh-1 and identified as nascent blood vessels may be nascent lymphatic vessels.

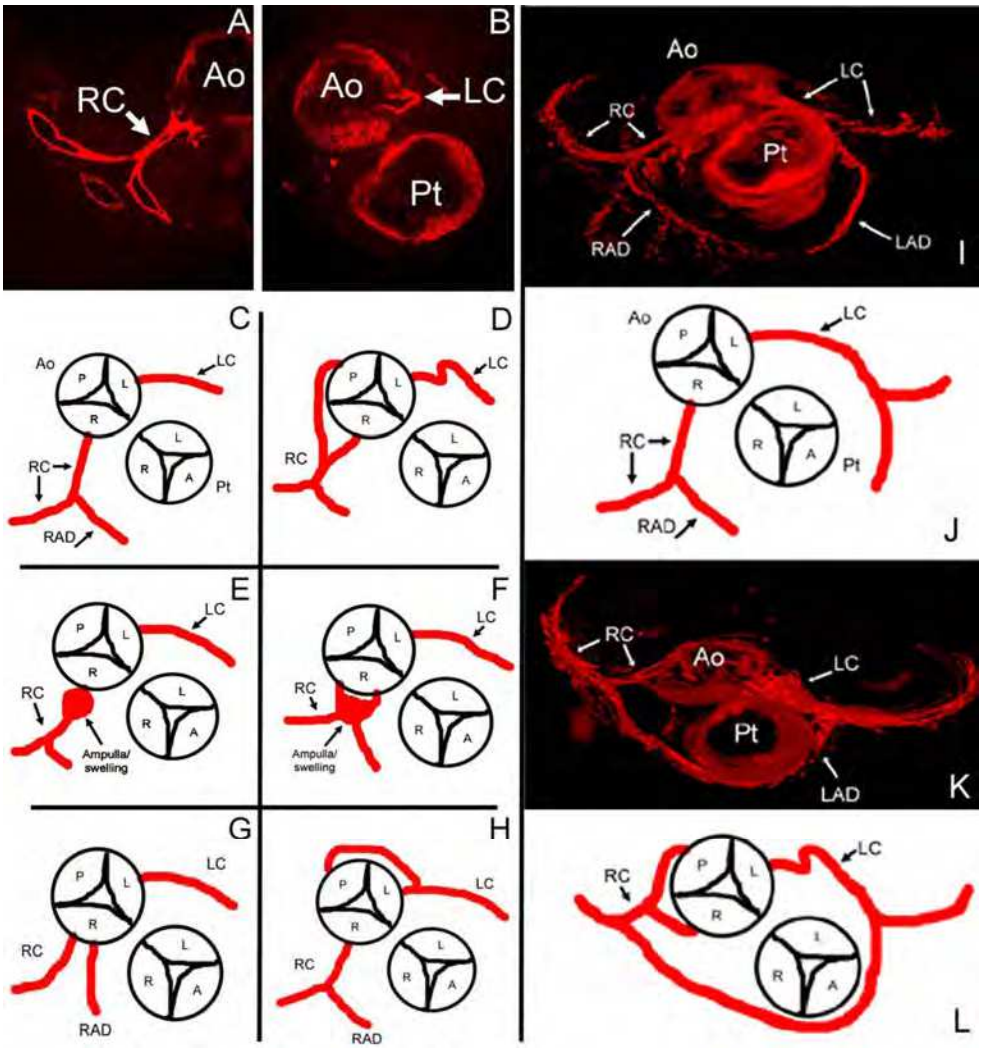


Fig. 3. **Hypoxia-induced defects in coronary vessels.** Transverse sections of stage 35 (ED 9) chicken embryo hearts stained with anti-a-smooth muscle actin-Cy3(A,B). A,B,C were incubated in 20.8% O2 (normoxic) conditions. D-H were incubated in 15% O2 (mildly hypoxic) for 4.5 days. 9 out of 10 embryos had defects that ranged from offset RC's near the posterior cusp (D,H), double RC's (F, G), & swollen RCs (E,F). LC's were tortuous and extended into abnormal positions (D). Ao = aorta, Pt =pulmonary trunk, RC = right coronary, LC = left coronary. [From (Wikenheiser et al., 2009)]

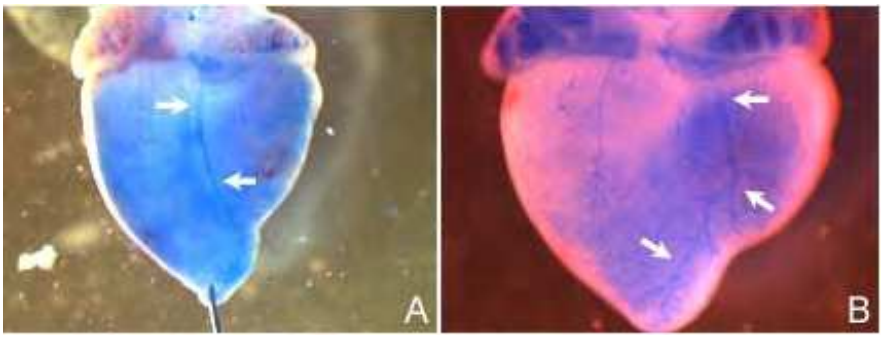


Fig. 4. Abnormal course of the posterior descending branch of the coronary artery after exposure to hypoxic conditions. The coronary vessels were filled by back-injection with blue ink into the aorta. The posterior vessel normally runs straight down the interventricular septum towards the apex (A; white arrows). This hypoxic embryo heart (B) had a vessel that diverged from this pathway along the sulcus before reaching the apex. These embryos were exposed to 15% O2 at stage 24-32, when coronary vessels are differentiating and remodeling, and harvested at stage 38.

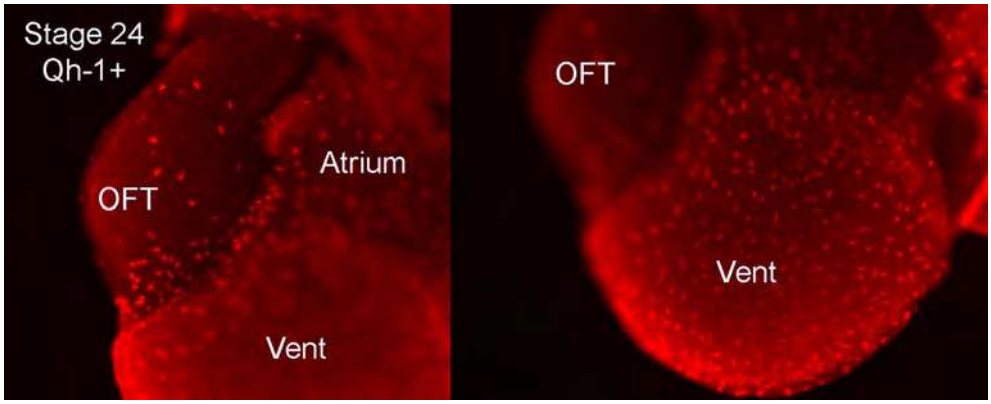


Fig. 5. Qh-1+ staining of vascular precursors cells in the epicardium of the quail heart (stage 24). At a stage prior to the formation of the four cardiac chambers, labeling using the antibody marker Qh-1 for endothelial cells and precursors revealed an evenly spaced set of cells covering the ventricular (Vent) and atrial (Atrium) surfaces and parts of the outflow tract (OFT). Will of these cells become endothelial cells and into which endothelial lineage will they be incorporated, arterial, venous or lymphatic? Why are they so evenly scattered at this stage and why aren't many covering the OFT. Qh-1 labels lymphatic endothelial cells as well as blood vessel endothelial cells and hemangioblasts.

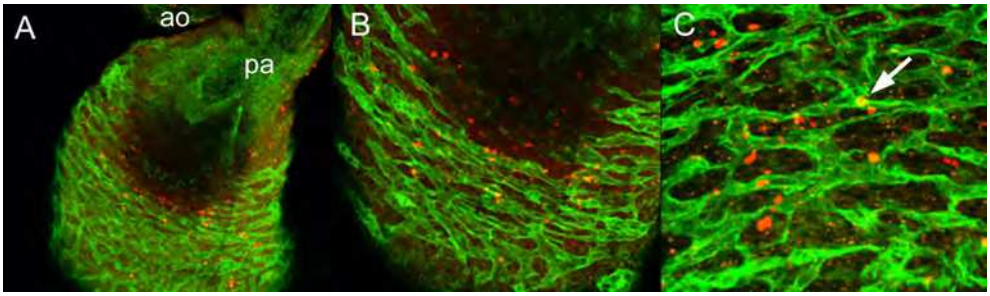


Fig. 6. Confocal microscopy of intact embryonic day E13.5 mouse heart double-labeled with an antibody to an endothelial marker PECAM (green) at the level of the outflow tract. The periarterial anatomoses, the primitive coronary vasculature surrounds the outflow tract. Lysotracker Red staining indicates that most of the apoptotic cells are within the myocardium not in the vasculature. The aorta (ao) and pulmonary artery (pa) are indicated at a low magnification (A, 100x) and higher magnifications are in (B, 200x) and (C, 400x) (Barbosky et al., 2006).

5. Origin of the lymphatics

Much less is known about the development of the lymphatics compared to the blood vessels within the heart. A number of reviews have been recently published regarding lymphangiogenesis (Albrecht and Christofori, 2011; Schulte-Merker, 2011; Witte et al., 2011). Transgenic mice have been created and markers for lymphatics have been identified that have made the study of lymphatic development much more accessible. The markers include antibodies to Prox-1, Lyve-1, Podoplanin, and VEGFR3. Unfortunately, these have limited use when used individually because, they are not exclusive to lymphatic endothelial cells especially in the embryo. They are useful in identifying bona fide lymphatic endothelial cells within the epicardium when used in combination (Karunamuni et al., 2010). It is quite clear that there are lymphatics in the epicardium of the adult heart but it is not known where they come from and exactly how they are distributed within the myocardium and endocardium. In view of their potential importance in cardiac homeostasis and disease, much more work is warranted.

6. Regulation of cardiac blood vessel development

Coronary vessels appear at particular stages of development and particular places within the heart. They can also grow and increase in density within the adult heart. The regulation of coronary vasculature is critical for homeostasis and as a response to stress. An understanding of what regulates these processes would be valuable in cardiotherapy. We expect that cardiac vessels are subject to some of the same regulatory strategies as other vessels in other parts of the body. However, the specific anatomy and demands of the heart suggest that there are likely to be cardiac-specific regulatory mechanisms to investigate.

The emergence of coronary vessels has been documented in quail using the antibody marker Qh1 that detects quail hemangioblasts/angioblasts and endothelial cells (Eralp, 2005; Kattan et al., 2004) and in mouse using PECAM and VEGFR2/Flk1 that detect endothelial cells [e.g.,(Tevosian et al., 2000)]. These early steps also appear to be choreographed such that precursors and endothelial cells assemble within the heart in a reproducible pattern that is

similar across species. The resulting mature avian coronary architecture is similar to that found in mammals (Sedmera and Watanabe, 2006; Tomanek et al., 2006a). The mechanisms by which these patterns are established have been speculated to be controlled by (1) the sequence of differentiation of cardiac regions, (2) mechanical factors (shear stress, cyclic stretch and strain), and (3) differential hypoxia.

The location of the largest arteries in the epicardium at the sulcus regions. Why do the largest coronary arteries lie within the epicardium and at the sulcus regions? This can be separated into two questions, why are the sulcus regions preferred and why at the level of the epicardium rather than in the myocardium? We hypothesize that the answer may lie in part in the differential hypoxia at these sites during the earliest stages of coronary vessel development.

Differential Hypoxia. The precursors of endothelial cells are subjected to many environmental influences. An obvious one is hypoxia. The lack of oxygen within cardiac tissues is likely the result of increased proliferation that results in tissues that are too thick for simple tissue diffusion to adequately provide oxygen to the cells. Increased activity of the constantly beating and maturing heart also depletes oxygen from myocardial tissues thus increasing activity would likely increase hypoxia.

We and others (e.g. (Nanka et al., 2008; Naňka et al., 2006; Sugishita et al., 2004c; Tomanek et al., 2003; Wikenheiser et al., 2006)) discovered by the use of hypoxia indicators that there is a pattern of differential hypoxia within the embryonic heart that may explain in part why vessels form or are more concentrated at particular places.

The spatiotemporal pattern of outflow tract (OFT) myocardial hypoxia as measured by the hypoxia indicator EF5 correlates with HIF-1a nuclear localization, cardiomyocyte apoptosis, and morphogenesis of the OFT in both avian and mouse embryos (Sugishita et al., 2004A, B, C; Barbosky et al., 2006; Wikenheiser et al., 2006). Incubation of embryos under hyperoxic conditions reduced tissue hypoxia, HIF-1a nuclear localization and cell death in the OFT myocardium and resulted in abnormal conotruncal morphologies. The expression of hypoxia regulated genes such as VEGFA and VEGFR2 was also affected by hypoxia and hyperoxia in the OFT myocardium. We concluded that relative hypoxia of the chicken embryo OFT at that particular stage was critical to normal morphogenesis. The hypoxia peak also correlated with when coronary vessel precursors accumulated around the proximal OFT in both chicken and mouse (Fig. 6) and when endothelial precursors invade the OFT myocardium (Barbosky et al., 2006; Rothenberg et al., 2002; Wessels and Perez-Pomares, 2004). These findings supported a spatiotemporal correlation between microenivornmental tissue hypoxia, HIF-1a nuclear localization, accumulation of endothelial precursors, and myocardial invasion of endothelial precursors and vessels.

In addition to the OFT, the hypoxia indicator EF5 bound to other cardiac regions that correspond to sites of coronary vessel formation. Using EF5, we determined that the chicken OFT myocardium was one of the most hypoxic cardiac tissues with a peak of EF5 staining intensity at the end of ventricular septation (Sugishita et al., 2004a; Sugishita et al., 2004c). Our subsequent studies (Wikenheiser et al., 2006) revealed additional intensely EF5-positive cardiac regions at intriguing sites that included myocardial regions of the atrial wall, the atrioventricular junction (AVJ), and the interventricular septum (IVS) (Fig 2). These regions were also positive for nuclear-localized HIF-1a suggesting that HIF-1-induced transcriptional regulation may be active at these sites. Many of the intensely EF5+ myocardial regions corresponded to sites where the major vessels of the coronary vasculature will eventually develop. These findings led us to the hypothesis that myocardial hypoxia at specific sites may

induce a level of HIF-1a mediated transcriptional activation that regulates gene expression critical to differentiation and organization of the coronary vasculature. The differential hypoxic microenvironments of the heart would provide a template for coronary vessel patterning.

Hypoxia Inducible Factors. An important and well-studied set of hypoxia sensitive transcription factors are the Hypoxia Inducible Factors (HIFs) of which HIF1a has received the most attention. Many cellular responses to hypoxic stress are regulated by the transcription factor HIF-1 (Semenza, 2001) a heterodimeric transcription factor composed of the constitutively expressed HIF-1b (ARNT) and the oxygen-sensitive HIF-1a. Under normoxic conditions, HIF-1a is degraded by ubiquitination. Under hypoxic conditions, HIF-1a escapes degradation, enters the nucleus, becomes a part of the heterdimer HIF-1, and binds to the CBP/p300 co-transactivator to form an active transcription complex that binds to hypoxia responsive elements (HREs) in promoter regions of many genes. In addition to this "canonical" function of HIF1a, this factor may participate in other pathways. For example HIF1a is known to bind to Notch and promote Notch signaling (Gustafsson et al., 2005) that is important in regulating differentiation of various cell types.

HIFs are critical for early development. The HIF-1a knockout mice die at E10.5 with abnormal morphogenesis of cardiac and other structures (Iyer et al., 1998; Ryan et al., 1998). The functional deletion of both HIF-1 and HIF-2 by expressing a dominant negative HIF2a led to embryonic lethality at E11.5. The conditional "knockout" of HIF-1a early in ventricular cardiomyocytes (Krishnan et al., 2008) and HIF-1 and HIF-2 by expression of a dominant negative HIF mutant in endothelial cells also resulted in early death [death by E11.5; (Licht, 2006)]. As coronary vessel development occurs at E10.5-13.5, the early death of these mice precluded determining the role of HIFs in coronary development by the use of these mice. A different strategy was required.

The HIF-1a gene was conditionally knocked out in cardiomyocytes of the left ventricle using the Cre-Lox technique with Cre expression driven by the *MLC2v* promoter (Huang et al., 2004). These mice survived with expected Mendelian frequencies, but with abnormal cardiomyocyte functions and a 15% reduction in vascularity of the left ventricle. These relatively mild effects suggested that HIF-1a is not required in cardiomyocytes for coronary vascular development, but may regulate its extent. However, compensation by HIF-2 (see below) or an alternate pathway is also possible. Another explanation is that the *MLC2v* promoter drives expression primarily in the left ventricle and at a late stage that may not knock out HIF-1a gene expression early and thoroughly enough to interfere with the myocardial paracrine signals for coronary development. These signals may have already been initiated prior to the knockout. Also the promoter may not have disabled the gene in the OFT myocardium or other sulcus regions where signals for early coronary development are likely to be critical. Their finding that smaller, recently developed vessels were more affected than larger vessels supports this idea. Another possibility is that VEGF and other important factors come from another source, the epicardium. Thus, this conditional knockout mouse study supported a role for myocardial HIF-1a on some aspects of coronary vessel organization but it is probably not the only tissue important in this function.

The role of HIFs in the endothelium has been less easily established by conditional knockout studies. Specific deletion of HIF-1a in endothelial cells affected adult tumor angiogenesis but had no detectable effect on embryonic vascular development (Tang et al., 2004). The specific deletion of HIF-2a in endothelial cells has resulted in variable results ranging from no effect on vasculature to yolk sac vascular defects (Compernolle et al., 2002; Duan et al., 2005; Peng, 2000; Scortegagna et al., 2003a; Scortegagna et al., 2003b; Tian et al., 1998). It takes the

functional deletion of both HIF-1 and HIF-2 by expressing a dominant negative HIF-2a to see an effect. This deletion as mentioned above caused a embryonic lethality at E11.5. These results support that HIFs can compensate for each other during development and may play a role in other cell types in addition to cardiomyocytes.

A distinguishing feature of the sulcus regions of the heart where the coronary vessels first begin to develop and where the largest coronary arteries end up is a high level of hypoxia and nuclear-localized HIF-1a as well as a high level of expression of HIF downstream genes in both the myocardium and epicardium (Fig. 7). Therefore microenvironmental hypoxia may promote steps in early coronary vessel development in a region-specific pattern by enhancing HIF function in the epicardium as well as the myocardium.

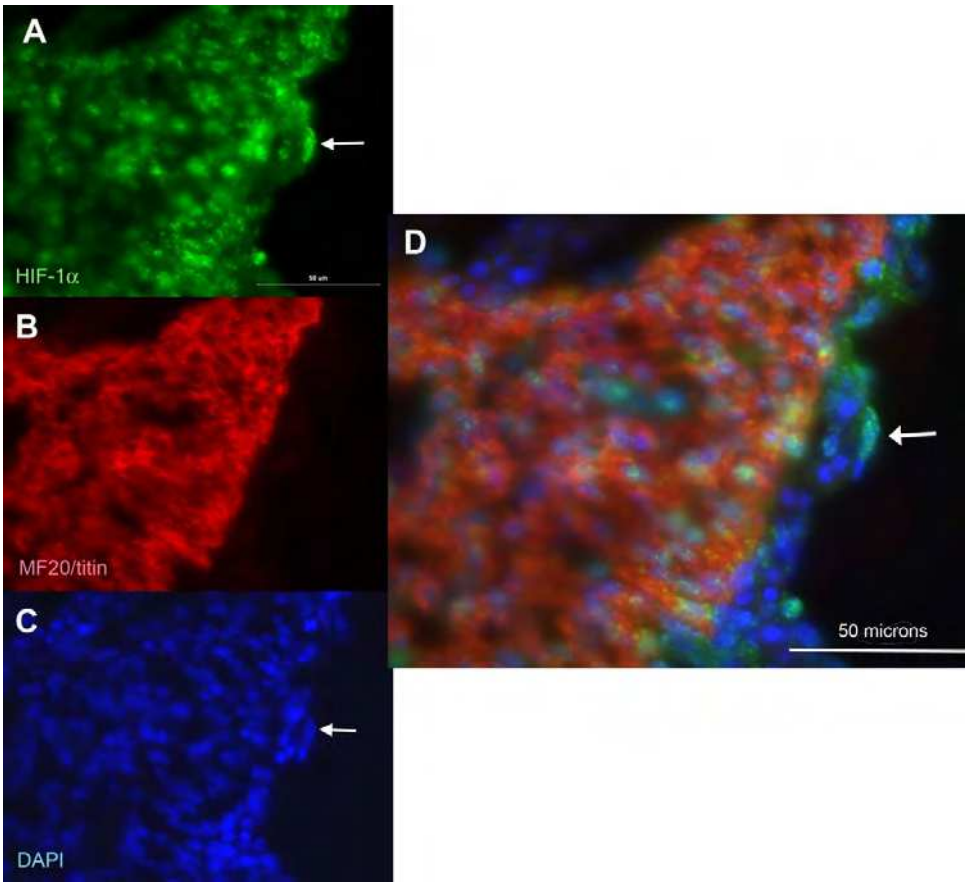


Fig. 7. HIF1a expressed in the epicardium. At the sulcus region of the ventricular apex were epicardial mesothelial cells with nuclear localized HIF1a staining (A, green). The myocardium is delineated using cardiomyocyte markers anti-MF20 and anti-titin (B, red). All nuclei are labeled with DAPI (C, blue). A composite overlay of all three colors (D) indicates an example of where HIF1a is expressed in the nuclei of an epicardial mesothelial cell. The same cell is delineated with a white arrow in A,C,and D.

Molecular factors in early steps of coronary vessel development. Factors that may be important for epicardial EMT and differentiation of these cells have been identified. Explant studies point to FGF (fibroblast growth factor) 2 and 7, VEGF(vascular endothelial growth factor), and EGF (epidermal growth factor) as inducers of EMT with TGFb's (transforming growth factor betas) acting surprisingly as inhibitors of EMT (Morabito et al., 2001). TGFb's act as enhancers of EMT in endocardial cushions. Thus the set of factors that control epicardial EMT resemble the set that control endocardial EMT but differ in their outcomes. These factors are expressed, as detected by immunohistology, within the embryonic epicardium and/or the myocardium. Though some factors involved in EMT and subsequent steps in epicardial cell differentiation have been identified, the mechanism that drives their expression in particular patterns, sites and stages is not fully understood.

Some of the same growth factors also influence the formation of tubes by endothelial precursors. These include VEGF family members and FGFs (Chen et al., 1994; Tomanek, 2006; Tomanek et al., 2008; Tomanek et al., 2002; Tomanek et al., 2006b; Tomanek et al., 2001). A gradient of VEGF expression across the ventricular wall has been observed in embryonic hearts that positively correlates with the level of hypoxia as detected by hypoxia indicators and HIF-1a nuclear localization (Sugishita et al., 2004b; Wikenheiser et al., 2006) and the density of vascular tubes (Tomanek et al., 2001). FGFs are also found in this gradient pattern and ectopic FGF expression caused abnormal coronary patterning within the ventricular wall (Pennisi and Mikawa, 2005). FGF family members are increased in their expression and secreted under hypoxic conditions in other systems (Berger et al., 2003; Moeller et al., 2004). Many factors important in coronary vessel formation are known to be hypoxia inducible and/or controlled directly or indirectly by HIF-1 in other systems.

In summary, a distinguishing feature of the sulcus regions of the heart where the coronary vessels first begin to develop and where the largest coronary arteries end up is a high level of hypoxia and nuclear-localized HIF-1a as well as a high level of expression of HIF downstream genes in both the myocardium and epicardium (Fig. 7).

Abnormally high HIF-1 activity results in abnormal coronary vessel development. If hypoxia via HIFs are an important signal of coronary vessels development, the expectation would be that altering the hypoxic microenvironment would alter coronary vascular development. There is evidence from several studies that this is the case.

Overactive HIF-1 transcriptional activity in the Cited2 knockout mouse results in coronary vascular abnormalities that can be rescued by reducing HIF-1a gene dosage. Cited2, a transcription factor that is regulated by HIF-1 transcriptional activity, down-regulates HIF-1 dependent gene expression in vitro (Bhattacharya et al., 1999)(Bhattacharya et al., 1999). Structural studies (Dames et al., 2002; Freedman et al., 2002; Semenza, 2002) indicated that Cited2 could act as an efficient competitor to HIF-1a binding to CBP/P300 and thus serve as an inhibitor of HIF-1 transcriptional activation. Defects of the Cited2-/- mouse, supported this role for Cited2 in vivo. Cited-/- mice had abnormally high HIF-1 transcriptional activation in the heart and defects in regions that are particularly hypoxic, including the OFT and IVS (Yin et al., 2002). The mRNA levels of HIF-1-regulated genes, VEGF, PGK and Glut-1, were elevated in Cited-/- embryonic hearts. Levels of secreted VEGF were also higher in fibroblast cultures from Cited2-/- as compared to those from Cited+/+ embryos. Vessels in the myocardium of Cited2-/- embryos were larger and leakier resembling vessels developing with VEGF overexpression (Xu et al., 2007). Reducing the gene dosage of HIF-1a in Cited2-/- mice by mating with the HIF-1a knockout line, partially rescued the cardiac defects (Xu et al., 2007). The coronary vasculature and VEGF mRNA transcription were also

closer to normal. These results suggested that hypoxia in embryonic heart tissues induces HIF-1 activity to support normal coronary vascular development, but inappropriately high levels of HIF-1 activity have a detrimental effect on coronary vascular and cardiac development. We could not conclude the cell type requirement for HIF-1a and Cited2 using these global Cited2 and HIF-1 knockout mice,

Hypoxic and hyperoxic conditions during embryogenesis alter coronary artery architecture. Regimens of hypoxia (15% O2) and hyperoxia compatible with long-term embryo survival caused coronary artery anomalies in chicken embryos (Wikenheiser et al., 2009)(Fig. 3,4). Regions with relatively high levels of microenvironmental hypoxia within the heart correlated with the expression of HIF-1 nuclear localization and HIF downstream genes at sulcus regions where the epicardium is thickest and where the largest arteries develop. Experimentally increasing the level of hypoxia caused a broadening in the regions of HIF-1a nuclear localized expression within the myocardium and epicardium, abnormal expression of HIF-1 downstream genes, thickening of the epicardium, disruption of the patterns of the early precursors of the coronaries and coronary artery anomalies. The epicardial cells at these sites have a very specific expression of nuclear-localized HIF-1a and N1ICD that indicates that in addition to the paracrine effects of HIFs from the adjacent myocardium, there may be a cell autonomous role for HIF and Notch in the embryonic epicardium (Yang et al., 2009).

Another consideration is the role that normal or abnormal vasculature can play in directing development of other tissues. Vascular development is intimately involved in the growth and development of the liver and pancreas from early inductive events (Bahary and Zon, 2001; Lammert et al., 2001; Lammert et al., 2003; Matsumoto et al., 2001). Evidence also supports the influence of coronary precursors and vessels on myocardial development and function and vice versa (Manner et al., 2001). For example, Purkinje fiber differentiation in avian systems appears to be controlled by factors from the coronary endothelium (Gourdie et al., 1998). *Thus, understanding the factors that regulate normal coronary vascular development is critical to a general understanding of embryonic and adult cardiac diseases and diseases of other tissues.*

7. Regulating the coronary vessels within the adult heart for cardiotherapy

The adult heart can adapt to stress by hypertrophy and vascular growth/remodeling (Bernardo et al., 2010; Weeks KL and McMullen JR, 2011). Physiological or adaptive responses, as in the case of the exercised trained athelete's heart, are characterized by balanced changes in both the cardiomyocytes and the vasculature. Negative consequences arise when these responses are not coordinated, as in the case of prolonged hypertension, and can lead to heart failure, arrhythmia, and death. The physiological and pathological responses overlap in some respects, especially in the early stages of adaptation. However, there are distinct characteristics to the physiological and pathological responses that have been revealed by studies in humans and rodent models (Boström P et al., 2010; Pavlik G et al., 2010; Weeks KL and McMullen JR, 2011). These differences include the degree of vascularization of the myocardium with the physiological response leading to increased vascularization.

Vascular development in the adult heart. Angiogenesis within the myocardium is not limited to developmental stages, but may occur when the mature heart is challenged by enhanced loading conditions or during hypoxia or ischemia. Angiogenesis in the setting of

the adult heart can be regulated by hypoxia-induced responses through HIF-1a, that can directly regulate endothelial cell activity and increase capillary density (Pugh CW and Ratcliffe PJ, 2003). Another mechanism is associated with a general stress response following acute hemodynamic overload or hypertrophy induced by exercise or pregnancy (Hilfiker-Kleiner D et al., 2005; Jacobs TB et al., 1984; Sano M et al., 2007; Waters RE et al., 2004). This response would allow the myocardium to coordinate an increase in microvessels with an ensuing hypertrophic response. This load-induced angiogenic response would also protect the heart from developing ischemia, possibly delaying decompensation. That HIF-1α was also activated by mechanical overload (Kim CH et al., 2002) suggests that there is some overlap in the mechanisms.

7.1 Myocardial hypertrophy and angiogenesis

Cardiac hypertrophy is associated with upregulation of VEGF in the myocardium (Izumiya Y et al., 2006). VEGF is required to maintain myocardial capillary density and reductions in the vascular bed are associated with the transition from compensatory hypertrophy to failure (Anversa P et al., 1986; Hudlicka O et al., 1992). In contrast, cardiac growth associated with development, nutritional input, or vigorous exercise is associated with maintained or increased capillary density (Hudlicka O et al., 1992; Shiojima I et al., 2002). Thus, the availability of VEGF may play a role in determining whether the phenotype of the growing heart is "physiological" or "pathological" (Shiojima I et al., 2005). In addition to the VEGF family, bFGF and angiopoietins play major roles in myocardial vascularization (Tomanek RJ et al., 1998; Visconti RP et al., 2002).

It was recently demonstrated that angiogenesis can induce myocardial hypertrophy even in the absence of a hemodynamic stimulus (Tirziu D et al., 2007). In this study an increase in the size of the vascular bed resulted in increased cardiac mass and myocardial hypertrophy paralleled by increased cardiac performance. This points out a complex role played by the endothelium of the heart (and likely other organs) that involves not only participation in a stress response but also regulation of the organ's size. The increase in heart size ceased once the vascular density returned to normal levels (Tirziu D et al., 2007).

Several important transcription factors have been studied for their ability to regulate the coronary vasculature in the adult mouse. These include GATA4 and CEBPb. The coronary vasculature within the myocardium increases in density when GATA4 expression is up-regulated (Heineke et al., 2007), or when CEBPb is down-regulated (Boström P et al., 2010) These vascular changes are also accompanied by improvement in function and survival when mice are subjected to a model of myocardial infarction. While effects on the coronary vasculature are not the only positive changes, this change is probably very important in providing coronary reserve during stresses. These promising preclinical findings suggest that targeting ways to regulate the transcription factors may be important in finding strategies for cardioprotection via increased vascularization.

If it is possible to find the pathways that lead to the morphology, physiology, and gene expression that resemble those of a physiological rather than a pathological response, that knowledge may allow us to coordinate a host of beneficial responses for the heart that would protect it from ischemic insults.

The adult epicardium has recently been shown to act as a paracrine source of factors that increases vessel density and benefits the health of cardiomyocytes (Zhou et al., 2011).

Preclinical studies to develop potential therapies and expected challenges. To prevent or alleviate effects of heart disease including myocardial infarction and heart failure, it would

be beneficial to increase the density of the vasculature and therefore perfusion of the myocardium. This increase would have to be controlled and the right mix of vessels would have to be formed in the right place at the right time. This enhancement of the coronary vasculature could be encouraged prior to surgery, or in early stages of coronary artery disease when a coronary occlusion is first noted or when early signs of pathological hypertrophy are identified. In cases of some anomalous coronaries it would be beneficial to encourage collateralization in a controlled way. An ability to promote and nudge coronary vessel development at the right time and place could benefit the outcome of many cardiac diseases.

Gene therapy. Current pharmacologic therapy for ischemic heart disease is limited by several issues including patient compliance and side effects of the medications (Lavu et al., 2011). Also a significant population of patients do not respond to the best medical therapy available. Gene therapy may be a promising alternative that is currently being investigated. Gene therapy with isoforms of growth factors such as Vascular Endothelial Growth Factor A (VEGFA) , Fibroblast Growth Factor (FGF) and Hepatocyte Growth Factor (HGF) induces angiogenesis, decreases apoptosis and leads to protection in the ischemic heart. Stem cell therapy combined with gene therapy promotes myogenesis in animal models of myocardial ischemia. Gene therapy that induces the expression of antioxidants, eNOS, SP, mitogen activated protein kinase and other anti-apoptotic proteins have also been shown to be beneficial in animal models. Clinical trials currently show mixed results and the interpretation of the results are controversial, but some of the therapies appear to be safe.

Vascular endothelial growth factor (VEGF) gene therapy: VEGFA is a highly investigated growth factor that induces angiogenesis in the ischemic heart. Isoforms of VEGFA bind to specific receptors on endothelial cells and play an essential role in angiogenesis (Hao et al., 2007). VEGFA-165 (VEGFA isoform) gene therapy using plasmids in rats (Dong et al., 2009; Yockman et al., 2009) or through non-viral delivery systems in rabbits (Aoki et al., 2000) induces significant neovascularization and improves fractional shortening after modeling myocardial infarction (MI).

Concerns with angiogenic therapies exist because VEGFA is a powerful factor whose expression level must be tightly regulated in isoform expression, timing and location. Deviation from normal levels are known to cause pathology in animal models [e.g. (Autiero et al., 2005; Carmeliet et al., 1996; Flamme et al., 1995; Kaner et al., 2000)]. Novel gene constructs have been created that would allow the gene expression to be responsive to the cellular environments. For example, some constructs allow VEGFA levels to be increased specifically during cardiac ischemia.

(Lee et al., 2003; Su et al., 2002) and the treated animals have reduced infarct size and increased angiogenesis (Dong et al., 2009; Yockman et al., 2009).

Hepatocyte growth factor gene therapy: Human Hepatocyte Growth Factor (hHGF) gene therapy induced angiogenesis in rats and dogs after experimental myocardial infarction (MI) and also improved cardiac function (Ahmet et al., 2002; Ahmet et al., 2003; Cho et al., 2008; Jayasankar et al., 2003; Jin et al., 2004; Li et al., 2003; Taniyama et al., 2002; Yang et al., 2007; Yang et al., 2010). HGF gene therapy combined with a novel gene transfection strategy that looks promising in a rat model of MI (Ahmet et al., 2003) is currently being evaluated in clinical trials.

Fibroblast growth factor gene therapy: Fibroblast growth factors FGF-1 and FGF-2 are known to promote endothelial cell proliferation and formation of tube-like endothelial structures in various pre-clinical models. FGF-2 gene therapy has been shown to improve

arteriogenesis and left ventricular (LV) function in a model of chronic ischemia in pigs (Heilmann et al., 2002; Horvath et al., 2002).

Angiogenesis through genetically modified cells: Genetically modified cells can be used to express the transgene of choice and lead to increased levels of the desired proteins in target tissues such as the heart. One obvious potential benefit of this strategy is its ability to activate precursor cells to differentiate into cardiac phenotypes, incorporate into the myocardium and prevent or alleviate harmful cardiac remodeling (Atkins et al., 1999)[29]. Vascular smooth muscle cells (VSMCs) modified to overexpress VEGF, when administered by the intra-coronary route in an intermittent repetitive LAD occlusion model increased collateral circulation in the ischemic heart (Hattan et al., 2004). Fibroblasts, modified to overexpress bFGF gene, when administered by coronary injections in a swine model of chronic ischemia led to improved collateral formation and myocardial contraction as measured by coronary angiography and electromechanical mapping (Ninomiya et al., 2003). Some advantages of mesenchymal stem cells (MSCs) are their ability to be transduced by vectors easily, ability to be delivered systemically and their capacity to home in to damaged tissues. MSCs also possess low immunogenicity and hence can be used allogenically (Ninomiya et al., 2003).

8. Overall summary

There are still many puzzling aspects of cardiac vessel development and anatomy that require further investigation. Fundamental questions regarding lymphangiogenesis in the heart may now be approachable with techniques and reagents currently available and already reveal surprising and puzzling findings. It is still unknown how coronary vessel anomalies develop. Discussions continue on the best way to detect them clinically and how to proceed once detected. Experiments on the developmental biology of coronary vessel development in genetically engineered mice have led to promising leads towards reducing or alleviating the consequences of myocardial infarction and heart disease so as to slow or prevent the progression to heart failure and death. By understanding the mechanisms that control coronary vessel localization and density, it may be possible to increase coronary vasculature with therapies even when exercise is not an option as a preventative measure to reduce or prevent myocardial ischemic damage during surgery or for individuals with a family history of heart disease.

9. Abbreviations

ALCAPA	Anomalous Left Coronary Artery from Pulmonary Artery
ASD	Atrial Septal Defect
AV	Atrioventricular
BMP	Bone Morphogenetic Protein
CAD	Coronary Artery Disease
CF	Circumflex Artery
EGF	Endothelial Growth Factor
EMT	Epithelial Mesenchymal Transition
EPDC	Epicardial Derived Cells
FGF	Fibroblast Growth Factor
FOG	Friend of GATA

HIF	Hypoxia Inducible Factor
HRE	Hypoxia Responsive Elements
LAD	Left Anterior Descending Coronary Artery
MI	Myocardial Infarction
OFT	Outflow Tract
PDA	Posterior Descending Artery
PE	Proepicardium
PEO	Proepicardial Organ
RCA	Right Coronary Artery
TGF	Transforming Growth Factor
VEGF	Vascular Endothelial Growth Factor
VSD	Ventricular Septal Defect

10. Acknowledgements

The authors thank Laura M. Bock, BFA, Medical Illustrator (laurabock.biomed@gmail.com) for artistic contributions, and Dr. Robert J. Tomanek for inspiration, encouragement, and critical discussions. NIH Grant funding (ARRA) from NHLBI HL091171 (M.W.) and AHA Fellowship funding to Ganga Karunamuni

11. References

Ahmet, I., Sawa, Y., Iwata, K., Matsuda, H., 2002. Gene transfection of hepatocyte growth factor attenuates cardiac remodeling in the canine heart: A novel gene therapy for cardiomyopathy. J Thorac Cardiovasc Surg. 124, 957-63.

Ahmet, I., Sawa, Y., Yamaguchi, T., Matsuda, H., 2003. Gene transfer of hepatocyte growth factor improves angiogenesis and function of chronic ischemic myocardium in canine heart. Ann Thorac Surg. 75, 1283-7.

Albrecht, I., Christofori, G., 2011. Molecular mechanisms of lymphangiogenesis in development and cancer. Int J Dev Biol. 55, 483-94.

Allen, H. D. D., D.J.;Shaddy,R.D. RD Shaddy; Feltes,T.F., 2007. Moss and Adams' Heart Disease in Infants, Children, and Adolescents. Lippincott, Williams & Wilkins.

Anversa P, Beghi C, Kikkawa Y, Olivetti G, C. R., 1986. Myocardial infarction in rats. Infarct size, myocyte hypertrophy, and capillary growth. Circ. Res. 58, 26-37.

Aoki, M., Morishita, R., Taniyama, Y., Kida, I., Moriguchi, A., Matsumoto, K., Nakamura, T., Kaneda, Y., Higaki, J., Ogihara, T., 2000. Angiogenesis induced by hepatocyte growth factor in non-infarcted myocardium and infarcted myocardium: up-regulation of essential transcription factor for angiogenesis, ets. Gene Ther. 7, 417-27.

Atkins, B. Z., Hueman, M. T., Meuchel, J., Hutcheson, K. A., Glower, D. D., Taylor, D. A., 1999. Cellular cardiomyoplasty improves diastolic properties of injured heart. J Surg Res. 85, 234-42.

Autiero, M., Desmet, F., Claes, F., Carmeliet, P., 2005. Role of neural guidance signals in blood vessel navigation. Cardiovascular Research. 65, 629-638.

Bahary, N., Zon, L. I., 2001. Development. Endothelium--chicken soup for the endoderm. Science. 294, 530-1.

Barbosky, L., Lawrence, D. K., Karunamuni, G., Wikenheiser, J. C., Doughman, Y. Q., Visconti, R. P., Burch, J. B., Watanabe, M., 2006. Apoptosis in the developing mouse heart. Dev Dyn. 235, 2592-602.

Berger, A. P., Kofler, K., Bektic, J., Rogatsch, H., Steiner, H., Bartsch, G., Klocker, H., 2003. Increased growth factor production in a human prostatic stromal cell culture model caused by hypoxia. Prostate. 57, 57-65.

Bernardo, B. C., Weeks, K. L., Pretorius, L., McMullen, J. R., 2010. Molecular distinction between physiological and pathological cardiac hypertrophy: experimental findings and therapeutic strategies. Pharmacol Ther. 128, 191-227.

Bhattacharya, S., Michels, C. L., Leung, M. K., Arany, Z. P., Kung, A. L., Livingston, D. M., 1999. Functional role of p35srj, a novel p300/CBP binding protein, during transactivation by HIF-1. Genes Dev. 13, 64-75.

Boktor, M., Mansi, I. A., Troxclair, S., Modi, K., 2009. Association of myocardial bridge and Takotsubo cardiomyopathy: a case report and literature review. South Med J. 102, 957-60.

Boström P, Mann N, Wu J, Quintero PA, Plovie ER, Panáková D, Gupta RK, Xiao C, MacRae CA, Rosenzweig A, Spiegelman BM, 2010. C/EBPβ controls exercise-induced cardiac growth and protects against pathological cardiac remodeling. Cell. 143, 1072-1083.

Bourassa, M. G., Butnaru, A., Lesperance, J., Tardif, J. C., 2003. Symptomatic myocardial bridges: overview of ischemic mechanisms and current diagnostic and treatment strategies. J Am Coll Cardiol. 41, 351-9.

Cai, C.-L., Martin, J. C., Sun, Y., Cui, L., Wang, L., Ouyang, K., Yang, L., Bu, L., Liang, X., Zhang, X., Stallcup, W. B., Denton, C. P., McCulloch, A., Chen, J., Evans, S. M., 2008. A myocardial lineage derives from Tbx18 epicardial cells. Nature. 454, 104-108.

Carmeliet, P., Ferreira, V., Breier, G., Pollefeyt, S., Kieckens, L., Gertsenstein, M., Fahrig, M., Vandenhoeck, A., Harpal, K., Eberhardt, C., Declercq, C., Pawling, J., Moons, L., Collen, D., Risau, W., Nagy, A., 1996. Abnormal blood vessel development and lethality in embryos lacking a single VEGF allele. Nature. 380, 435-9.

Cheitlin, M. D., MacGregor, J., 2009. Congenital anomalies of coronary arteries: role in the pathogenesis of sudden cardiac death. Herz. 34, 268-79.

Chen, Y., Torry, R. J., Baumbach, G. L., Tomanek, R. J., 1994. Proportional arteriolar growth accompanies cardiac hypertrophy induced by volume overload. Am J Physiol. 267, H2132-7.

Chiam, P. T., Ruiz, C. E., 2011. Percutaneous transcatheter mitral valve repair: a classification of the technology. JACC Cardiovasc Interv. 4, 1-13.

Cho, K. R., Choi, J. S., Hahn, W., Kim, D. S., Park, J. S., Lee, D. S., Kim, K. B., 2008. Therapeutic angiogenesis using naked DNA expressing two isoforms of the hepatocyte growth factor in a porcine acute myocardial infarction model. Eur J Cardiothorac Surg. 34, 857-63.

Compernolle, V., Brusselmans, K., Acker, T., Hoet, P., Tjwa, M., Beck, H., Plaisance, S., Dor, Y., Keshet, E., Lupu, F., Nemery, B., Dewerchin, M., Van Veldhoven, P., Plate, K., Moons, L., Collen, D., Carmeliet, P., 2002. Loss of HIF-2α and inhibition of VEGF impair fetal lung maturation, whereas treatment with VEGF prevents fatal respiratory distress in premature mice. Nature Medicine.

Compton, L. A., Potash, D. A., Mundell, N. A., Barnett, J. V., 2006. Transforming growth factor-β induces loss of epithelial character and smooth muscle cell differentiation in epicardial cells. Developmental Dynamics. 235, 82-93.

Dames, S. A., Martinez-Yamout, M., De Guzman, R. N., Dyson, H. J., Wright, P. E., 2002. Structural basis for Hif-1 alpha /CBP recognition in the cellular hypoxic response. Proc Natl Acad Sci U S A. 99, 5271-6.

Dong, H., Wang, Q., Zhang, Y., Jiang, B., Xu, X., Zhang, Z., 2009. Angiogenesis induced by hVEGF165 gene controlled by hypoxic response elements in rabbit ischemia myocardium. Exp Biol Med (Maywood). 234, 1417-24.

Duan, L. J., Zhang-Benoit, Y., Fong, G. H., 2005. Endothelium-intrinsic requirement for Hif-2alpha during vascular development. Circulation. 111, 2227-32.

Eckart, R. E., Jones, S. O. t., Shry, E. A., Garrett, P. D., Scoville, S. L., 2006a. Sudden death associated with anomalous coronary origin and obstructive coronary disease in the young. Cardiol Rev. 14, 161-3.

Eckart, R. E., Scoville, S. L., Shry, E. A., Potter, R. N., Tedrow, U., 2006b. Causes of sudden death in young female military recruits. Am J Cardiol. 97, 1756-8.

Epstein, S. E., Cannon, R. O., 3rd, Talbot, T. L., 1985. Hemodynamic principles in the control of coronary blood flow. Am J Cardiol. 56, 4E-10E.

Eralp, I., 2005. Coronary Artery and Orifice Development Is Associated With Proper Timing of Epicardial Outgrowth and Correlated Fas Ligand Associated Apoptosis Patterns. Circulation Research. 96, 526-534.

Eralp, I., Lie-Venema, H., DeRuiter, M. C., van den Akker, N. M., Bogers, A. J., Mentink, M. M., Poelmann, R. E., Gittenberger-de Groot, A. C., 2005. Coronary artery and orifice development is associated with proper timing of epicardial outgrowth and correlated Fas-ligand-associated apoptosis patterns. Circ Res. 96, 526-34.

Flamme, I., von Reutern, M., Drexler, H. C., Syed-Ali, S., Risau, W., 1995. Overexpression of vascular endothelial growth factor in the avian embryo induces hypervascularization and increased vascular permeability without alterations of embryonic pattern formation. Dev Biol. 171, 399-414.

Fontana, R. S., Edwards, J.E., 1962. Congenital Cardiac Disease: A Review of 357 Cases Studied Pathologically. W.B. Saunders, Philadelphia.

Freedman, S. J., Sun, Z. Y., Poy, F., Kung, A. L., Livingston, D. M., Wagner, G., Eck, M. J., 2002. Structural basis for recruitment of CBP/p300 by hypoxia-inducible factor-1 alpha. Proc Natl Acad Sci U S A. 99, 5367-72.

Frescura, C., Basso, C., Thiene, G., Corrado, D., Pennelli, T., Angelini, A., Daliento, L., 1998. Anomalous origin of coronary arteries and risk of sudden death: a study based on an autopsy population of congenital heart disease. Hum Pathol. 29, 689-95.

Frescura, C. B., C.;Thiene, G.;Corrado, D.;Pennelli, T.;Angelini, A.; Daliento, L., 1999. Anomalous origin of coronary arteries and risk of sudden death: a study based on an autopsy population of congenital heart disease. Hum Pathol. 29, 689-695.

Fulton, W. F. M., 1964. Morphology of the myocardial microcirculation. British Heart Journal. 26, 39-50.

Gensini, G. G., Digiorgi, S., Coskun, O., Palacio, A., Kelly, A. E., 1965. Anatomy of the Coronary Circulation in Living Man; Coronary Venography. Circulation. 31, 778-84.

George, J. M., Knowlan, D. M., 1959. Anomalous origin of the left coronary artery from the pulmonary artery in an adult. N Engl J Med. 261, 993-8.

Gilard, M., Mansourati, J., Etienne, Y., Larlet, J. M., Truong, B., Boschat, J., Blanc, J. J., 1998. Angiographic anatomy of the coronary sinus and its tributaries. Pacing Clin Electrophysiol. 21, 2280-4.

Gittenberger-de Groot, A. C., Vrancken Peeters, M. P., Bergwerff, M., Mentink, M. M., Poelmann, R. E., 2000. Epicardial outgrowth inhibition leads to compensatory mesothelial outflow tract collar and abnormal cardiac septation and coronary formation. Circ Res. 87, 969-71.

Gittenberger-de Groot, A. C., Winter, E. M., Poelmann, R. E., 2010. Epicardium-derived cells (EPDCs) in development, cardiac disease and repair of ischemia. J Cell Mol Med. 14, 1056-60.

Gourdie, R. G., Wei, Y., Kim, D., Klatt, S. C., Mikawa, T., 1998. Endothelin-induced conversion of embryonic heart muscle cells into impulse-conducting Purkinje fibers. Proc Natl Acad Sci U S A. 95, 6815-8.

Grace, R. R., Angelini, P., Cooley, D. A., 1977. Aortic implantation of anomalous left coronary artery arising from pulmonary artery. Am J Cardiol. 39, 609-13.

Gustafsson, M. V., Zheng, X., Pereira, T., Gradin, K., Jin, S., Lundkvist, J., Ruas, J. L., Poellinger, L., Lendahl, U., Bondesson, M., 2005. Hypoxia Requires Notch Signaling to Maintain the Undifferentiated Cell State. Developmental Cell. 9, 617-628.

Hakeem, A., Cilingiroglu, M., Leesar, M. A., 2010. Hemodynamic and intravascular ultrasound assessment of myocardial bridging: fractional flow reserve paradox with dobutamine versus adenosine. Catheter Cardiovasc Interv. 75, 229-36.

Hao, X., Mansson-Broberg, A., Grinnemo, K. H., Siddiqui, A. J., Dellgren, G., Brodin, L. A., Sylven, C., 2007. Myocardial angiogenesis after plasmid or adenoviral VEGF-A(165) gene transfer in rat myocardial infarction model. Cardiovasc Res. 73, 481-7.

Hattan, N., Warltier, D., Gu, W., Kolz, C., Chilian, W. M., Weihrauch, D., 2004. Autologous vascular smooth muscle cell-based myocardial gene therapy to induce coronary collateral growth. Am J Physiol Heart Circ Physiol. 287, H488-93.

Haugen, O. A., Ellingsen, C. L., 2007. [Coronary artery anomalies as a cause of sudden death in young people]. Tidsskr Nor Laegeforen. 127, 190-2.

Heilmann, C., von Samson, P., Schlegel, K., Attmann, T., von Specht, B. U., Beyersdorf, F., Lutter, G., 2002. Comparison of protein with DNA therapy for chronic myocardial ischemia using fibroblast growth factor-2. Eur J Cardiothorac Surg. 22, 957-64.

Heineke, J., Auger-Messier, M., Xu, J., Oka, T., Sargent, M. A., York, A., Klevitsky, R., Vaikunth, S., Duncan, S. A., Aronow, B. J., Robbins, J., Cromblehol, T. M., Molkentin, J. D., 2007. Cardiomyocyte GATA4 functions as a stress-responsive regulator of angiogenesis in the murine heart. Journal of Clinical Investigation. 117, 3198-3210.

Hilfiker-Kleiner D, Hilfiker A, Kaminski K, Schaefer A, Park JK, Michel K, Quint A, Yaniv M, Weitzman JB, Drexler H, 2005. Lack of JunD promotes pressure overload-induced apoptosis, hypertrophic growth, and angiogenesis in the heart. . Circulation. 112, 1470-1477.

Hiruma, T., Hirakow, R., 1989. Epicardial formation in embryonic chick heart: computer-aided reconstruction, scanning, and transmission electron microscopic studies. Am J Anat. 184, 129-38.

Ho, E., Shimada, Y., 1978. Formation of the epicardium studied with the scanning electron microscope. Dev Biol. 66, 579-85.

Horvath, K. A., Doukas, J., Lu, C. Y., Belkind, N., Greene, R., Pierce, G. F., Fullerton, D. A., 2002. Myocardial functional recovery after fibroblast growth factor 2 gene therapy as assessed by echocardiography and magnetic resonance imaging. Ann Thorac Surg. 74, 481-6; discussion 487.

Huang, Y., Hickey, R. P., Yeh, J. L., Liu, D., Dadak, A., Young, L. H., Johnson, R. S., Giordano, F. J., 2004. Cardiac myocyte-specific HIF-1alpha deletion alters vascularization, energy availability, calcium flux, and contractility in the normoxic heart. FASEB J. 18, 1138-40.

Huddleston, C. B., Balzer, D. T., Mendeloff, E. N., 2001. Repair of anomalous left main coronary artery arising from the pulmonary artery in infants: long-term impact on the mitral valve. Ann Thorac Surg. 71, 1985-8; discussion 1988-9.

Hudlicka O, Brown M, Egginton S, 1992. Angiogenesis in skeletal and cardiac muscle. Physiol. Rev. 72, 369-417.

Ishii, Y., Garriock, R. J., Navetta, A. M., Coughlin, L. E., Mikawa, T., 2010. BMP signals promote proepicardial protrusion necessary for recruitment of coronary vessel and epicardial progenitors to the heart. Dev Cell. 19, 307-16.

Iyer, N. V., Kotch, L. E., Agani, F., Leung, S. W., Laughner, E., Wenger, R. H., Gassmann, M., Gearhart, J. D., Lawler, A. M., Yu, A. Y., Semenza, G. L., 1998. Cellular and developmental control of O2 homeostasis by hypoxia-inducible factor 1 alpha. Genes Dev. 12, 149-62.

Izumiya Y, Shiojima I, Sato K, Sawyer DB, Colucci WS, Walsh K, 2006. Vascular endothelial growth factor blockade promotes the transition from compensatory cardiac hypertrophy to failure in response to pressure overload. . Hypertension. 47, 887-893.

Jacobs TB, Bell RD, McClements JD, 1984. Exercise, age and the development of the myocardial vasculature. . Growth. 48, 148-157.

Jayasankar, V., Woo, Y. J., Bish, L. T., Pirolli, T. J., Chatterjee, S., Berry, M. F., Burdick, J., Gardner, T. J., Sweeney, H. L., 2003. Gene transfer of hepatocyte growth factor attenuates postinfarction heart failure. Circulation. 108 Suppl 1, II230-6.

Jin, H., Wyss, J. M., Yang, R., Schwall, R., 2004. The therapeutic potential of hepatocyte growth factor for myocardial infarction and heart failure. Curr Pharm Des. 10, 2525-33.

Jin, Z., Berger, F., Uhlemann, F., Schroder, C., Hetzer, R., Alexi-Meskhishvili, V., Weng, Y., Lange, P. E., 1994. Improvement in left ventricular dysfunction after aortic reimplantation in 11 consecutive paediatric patients with anomalous origin of the left coronary artery from the pulmonary artery. Early results of a serial echocardiographic follow-up. Eur Heart J. 15, 1044-9.

Kaner, R. J., Ladetto, J. V., Singh, R., Fukuda, N., Matthay, M. A., Crystal, R. G., 2000. Lung overexpression of the vascular endothelial growth factor gene induces pulmonary edema. Am J Respir Cell Mol Biol. 22, 657-64.

Karunamuni, G., Yang, K., Doughman, Y. Q., Wikenheiser, J., Bader, D., Barnett, J., Austin, A., Parsons-Wingerter, P., Watanabe, M., 2010. Expression of Lymphatic Markers During Avian and Mouse Cardiogenesis. Anat Rec (Hoboken).

Kattan, J., Dettman, R. W., Bristow, J., 2004. Formation and remodeling of the coronary vascular bed in the embryonic avian heart. Dev Dyn. 230, 34-43.

Kim CH, Cho YS, Chun YS, Park JW, Kim MS, 2002. Early expression of myocardial HIF-1alpha in response to mechanical stresses: regulation by stretch-activated channels and the phosphatidylinositol 3-kinase signaling pathway. Circ. Res. 90, E25-E33.

Krishnan, J., Ahuja, P., Bodenmann, S., Knapik, D., Perriard, E., Krek, W., Perriard, J. C., 2008. Essential Role of Developmentally Activated Hypoxia-Inducible Factor 1 for Cardiac Morphogenesis and Function. Circulation Research. 103, 1139-1146.

Laifer, L. I., Weiner, B. H., 1991. Percutaneous transluminal coronary angioplasty of a coronary artery stenosis at the site of myocardial bridging. Cardiology. 79, 245-8.

Lammert, E., Cleaver, O., Melton, D., 2001. Induction of pancreatic differentiation by signals from blood vessels. Science. 294, 564-7.

Lammert, E., Cleaver, O., Melton, D., 2003. Role of endothelial cells in early pancreas and liver development. Mech Dev. 120, 59-64.

Lavu, M., Gundewar, S., Lefer, D. J., 2011. Gene therapy for ischemic heart disease. J Mol Cell Cardiol. 50, 742-50.

Lee, M., Rentz, J., Bikram, M., Han, S., Bull, D. A., Kim, S. W., 2003. Hypoxia-inducible VEGF gene delivery to ischemic myocardium using water-soluble lipopolymer. Gene Ther. 10, 1535-42.

Li, Y., Takemura, G., Kosai, K., Yuge, K., Nagano, S., Esaki, M., Goto, K., Takahashi, T., Hayakawa, K., Koda, M., Kawase, Y., Maruyama, R., Okada, H., Minatoguchi, S., Mizuguchi, H., Fujiwara, T., Fujiwara, H., 2003. Postinfarction treatment with an adenoviral vector expressing hepatocyte growth factor relieves chronic left ventricular remodeling and dysfunction in mice. Circulation. 107, 2499-506.

Licht, A. H., 2006. Inhibition of hypoxia-inducible factor activity in endothelial cells disrupts embryonic cardiovascular development. Blood. 107, 584-590.

Lie-Venema, H., van den Akker, N. M., Bax, N. A., Winter, E. M., Maas, S., Kekarainen, T., Hoeben, R. C., deRuiter, M. C., Poelmann, R. E., Gittenberger-de Groot, A. C., 2007. Origin, fate, and function of epicardium-derived cells (EPDCs) in normal and abnormal cardiac development. ScientificWorldJournal. 7, 1777-98.

Liebman, J., Hellerstein, H. K., Ankeney, J. L., Tucker, A., 1963. The Problem of the Anomalous Left Coronary Artery Arising from the Pulmonary Artery in Older Children. Report of Three Cases. N Engl J Med. 269, 486-94.

Loukas, M., Curry, B., Bowers, M., Louis, R. G., Jr., Bartczak, A., Kiedrowski, M., Kamionek, M., Fudalej, M., Wagner, T., 2006. The relationship of myocardial bridges to coronary artery dominance in the adult human heart. J Anat. 209, 43-50.

Luo, L., Kebede, S., Wu, S., Stouffer, G. A., 2006. Coronary artery fistulae. Am J Med Sci. 332, 79-84.

Manner, J., 1993. Experimental study on the formation of the epicardium in chick embryos. Anat Embryol (Berl). 187, 281-9.

Manner, J., Perez-Pomares, J. M., Macias, D., Munoz-Chapuli, R., 2001. The origin, formation and developmental significance of the epicardium: a review. Cells Tissues Organs. 169, 89-103.

Matsumoto, K., Yoshitomi, H., Rossant, J., Zaret, K. S., 2001. Liver organogenesis promoted by endothelial cells prior to vascular function. Science. 294, 559-63.

Mikawa, T., Fischman, D. A., 1992. Retroviral analysis of cardiac morphogenesis: discontinuous formation of coronary vessels. Proc Natl Acad Sci U S A. 89, 9504-8.

Miller, A. J., 1982. Lymphatics of the Heart. Raven Press, New York.

Moeller, B. J., Cao, Y., Vujaskovic, Z., Li, C. Y., Haroon, Z. A., Dewhirst, M. W., 2004. The relationship between hypoxia and angiogenesis. Semin Radiat Oncol. 14, 215-21.

Morabito, C. J., Dettman, R. W., Kattan, J., Collier, J. M., Bristow, J., 2001. Positive and negative regulation of epicardial-mesenchymal transformation during avian heart development. Dev Biol. 234, 204-15.

Mosher, P., Ross, J., Jr., McFate, P. A., Shaw, R. F., 1964. Control of Coronary Blood Flow by an Autoregulatory Mechanism. Circ Res. 14, 250-9.

Nahirney, P. C., Mikawa, T., Fischman, D. A., 2003. Evidence for an extracellular matrix bridge guiding proepicardial cell migration to the myocardium of chick embryos. Dev Dyn. 227, 511-23.

Nanka, O., Krizova, P., Fikrle, M., Tuma, M., Blaha, M., Grim, M., Sedmera, D., 2008. Abnormal Myocardial and Coronary Vasculature Development in Experimental Hypoxia. The Anatomical Record: Advances in Integrative Anatomy and Evolutionary Biology. 291, 1187-1199.

Naňka, O., Valášek, P., Dvořáková, M., Grim, M., 2006. Experimental hypoxia and embryonic angiogenesis. Developmental Dynamics. 235, 723-733.

Nesbitt, T., Lemley, A., Davis, J., Yost, M. J., Goodwin, R. L., Potts, J. D., 2006. Epicardial development in the rat: a new perspective. Microsc Microanal. 12, 390-8.

Neufeld, H. N., Schneeweiss, A., 1983. Coronary Artery Disease in Infants and Children. Lea & Febiger, Philadelphia.

Ninomiya, M., Koyama, H., Miyata, T., Hamada, H., Miyatake, S., Shigematsu, H., Takamoto, S., 2003. Ex vivo gene transfer of basic fibroblast growth factor improves cardiac function and blood flow in a swine chronic myocardial ischemia model. Gene Ther. 10, 1152-60.

Ogden, J. A., 1970. Congenital anomalies of the coronary arteries. Am J Cardiol. 25, 474-9.

Olivey, H. E., Compton, L. A., Barnett, J. V., 2004. Coronary vessel development: the epicardium delivers. Trends Cardiovasc Med. 14, 247-51.

Olivey, H. E., Mundell, N. A., Austin, A. F., Barnett, J. V., 2006. Transforming growth factor-β stimulates epithelial–mesenchymal transformation in the proepicardium. Developmental Dynamics. 235, 50-59.

Olivey, H. E., Svensson, E. C., 2010. Epicardial-myocardial signaling directing coronary vasculogenesis. Circ Res. 106, 818-32.

Park, M. K., 1988. Pediatric Cardiology for Practitioners. Year Book Medical Publishers, Inc., Boca Raton.

Parsons-Wingerter, P., McKay, T. L., Leontiev, D., Vickerman, M. B., Condrich, T. K., Dicorleto, P. E., 2006. Lymphangiogenesis by blind-ended vessel sprouting is concurrent with hemangiogenesis by vascular splitting. The Anatomical Record Part A: Discoveries in Molecular, Cellular, and Evolutionary Biology. 288A, 233-247.

Pavlik G, Major Z, Varga-Pintér B, Jeserich M, Kneffel Z, 2010. The athlete's heart Part I Acta Physiol. Hung. . 97, 337-353.

Pena, E., Nguyen, E. T., Merchant, N., Dennie, G., 2009. ALCAPA syndrome: not just a pediatric disease. Radiographics. 29, 553-65.

Peng, J., 2000. The transcription factor EPAS-1/hypoxia-inducible factor 2alpha plays an important role in vascular remodeling. Proceedings of the National Academy of Sciences. 97, 8386-8391.

Pennisi, D. J., Mikawa, T., 2005. Normal patterning of the coronary capillary plexus is dependent on the correct transmural gradient of FGF expression in the myocardium. Dev Biol. 279, 378-90.

Perez-Pomares, J. M., Phelps, A., Sedmerova, M., Wessels, A., 2003. Epicardial-like cells on the distal arterial end of the cardiac outflow tract do not derive from the proepicardium but are derivatives of the cephalic pericardium. Dev Dyn. 227, 56-68.

Pugh CW, Ratcliffe PJ, 2003. Regulation of angiogenesis by hypoxia: role of the HIF system. Nat. Med. 9, 677-684.

Que, J., Wilm, B., Hasegawa, H., Wang, F., Bader, D., Hogan, B. L., 2008. Mesothelium contributes to vascular smooth muscle and mesenchyme during lung development. Proc Natl Acad Sci U S A. 105, 16626-30.

Ratajska, A., Czarnowska, E., Ciszek, B., 2008. Embryonic development of the proepicardium and coronary vessels. Int J Dev Biol. 52, 229-36.

Rayman, H. C., 1737. Dissertation de vasis cordis propriis. Haller Bibl Anat. 2, 366.

Red-Horse, K., Ueno, H., Weissman, I. L., Krasnow, M. A., 2010. Coronary arteries form by developmental reprogramming of venous cells. Nature. 464, 549-53.

Reese, D. E., Mikawa, T., Bader, D. M., 2002. Development of the coronary vessel system. Circ Res. 91, 761-8.

Rothenberg, F., Hitomi, M., Fisher, S. A., Watanabe, M., 2002. Initiation of apoptosis in the developing avian outflow tract myocardium. Dev Dyn. 223, 469-82.

Ryan, H. E., Lo, J., Johnson, R. S., 1998. HIF-1 alpha is required for solid tumor formation and embryonic vascularization. EMBO J. 17, 3005-15.

Sano M, Minamino T, Toko H, Miyauchi H, Orimo M, Qin Y, Akazawa H, Tateno K, Kayama Y, Harada M, Shimizu I, Asahara T, Hamada H, Tomita S, Molkentin JD, Zou Y, Komuro I, 2007. p53-induced inhibition of Hif-1 causes cardiac dysfunction during pressure overload. . Nature. 446, 444-448.

Schamroth, C., 2009. Coronary artery fistula. J Am Coll Cardiol. 53, 523.

Schmitt, R., Froehner, S., Brunn, J., Wagner, M., Brunner, H., Cherevatyy, O., Gietzen, F., Christopoulos, G., Kerber, S., Fellner, F., 2005. Congenital anomalies of the coronary arteries: imaging with contrast-enhanced, multidetector computed tomography. Eur Radiol. 15, 1110-21.

Schulte-Merker, S., Sabine, A., Petrova, T.V., 2011. Lymphatic vascular mophogenesis in development, physiology, and disease. Journal Cell Biology. 193, 607-618.

Schwartz, M. L., Jonas, R. A., Colan, S. D., 1997. Anomalous origin of left coronary artery from pulmonary artery: recovery of left ventricular function after dual coronary repair. J Am Coll Cardiol. 30, 547-53.

Scortegagna, M., Ding, K., Oktay, Y., Gaur, A., Thurmond, F., Yan, L. J., Marck, B. T., Matsumoto, A. M., Shelton, J. M., Richardson, J. A., Bennett, M. J., Garcia, J. A., 2003a. Multiple organ pathology, metabolic abnormalities and impaired homeostasis of reactive oxygen species in Epas1(-/-) mice. Nature Genetics. 35, 331-340.

Scortegagna, M., Morris, M. A., Oktay, Y., Bennett, M., Garcia, J. A., 2003b. The HIF family member EPAS1/HIF-2 alpha is required for normal hematopoiesis in mice. Blood. 102, 1634-1640.

Sedmera, D., Watanabe, M., 2006. Growing the coronary tree: the quail saga. Anat Rec A Discov Mol Cell Evol Biol. 288, 933-5.

Semenza, G. L., 2001. HIF-1 and mechanisms of hypoxia sensing. Curr Opin Cell Biol. 13, 167-71.

Semenza, G. L., 2002. Physiology meets biophysics: visualizing the interaction of hypoxia-inducible factor 1 alpha with p300 and CBP. Proc Natl Acad Sci U S A. 99, 11570-2.

Shiojima I, Sato K, Izumiya Y, Schiekofer S, Ito M, Liao R, Colucci WS, Walsh K, 2005. Disruption of coordinated cardiac hypertrophy and angiogenesis contributes to the transition to heart failure. . J. Clin. Invest. 115, 2108-2118.

Shiojima I, Yefremashvili M, Luo Z, Kureishi Y, Takahashi A, Tao J, Rosenzweig A, Kahn CR, Abel ED, 37670–37677, W. K. J. B. C., 2002. Akt signaling mediates postnatal heart growth in response to insulin and nutritional status. J. Biol. Chem. 277.

Shirani, J., Roberts, W. C., 1993. Solitary coronary ostium in the aorta in the absence of other major congenital cardiovascular anomalies. J Am Coll Cardiol. 21, 137-43.

Smart, N., Bollini, S., Dube, K. N., Vieira, J. M., Zhou, B., Davidson, S., Yellon, D., Riegler, J., Price, A. N., Lythgoe, M. F., Pu, W. T., Riley, P. R., 2011. De novo cardiomyocytes from within the activated adult heart after injury. Nature. 474, 640-4.

Sridurongrit, S., Larsson, J., Schwartz, R., Ruiz-Lozano, P., Kaartinen, V., 2008. Signaling via the Tgf-beta type I receptor Alk5 in heart development. Dev Biol. 322, 208-18.

Su, H., Arakawa-Hoyt, J., Kan, Y. W., 2002. Adeno-associated viral vector-mediated hypoxia response element-regulated gene expression in mouse ischemic heart model. Proc Natl Acad Sci U S A. 99, 9480-5.

Sugishita, Y., Leifer, D. W., Agani, F., Watanabe, M., Fisher, S. A., 2004a. Hypoxia-responsive signaling regulates the apoptosis-dependent remodeling of the embryonic avian cardiac outflow tract. Dev Biol. 273, 285-96.

Sugishita, Y., Watanabe, M., Fisher, S. A., 2004b. The development of the embryonic outflow tract provides novel insights into cardiac differentiation and remodeling. Trends Cardiovasc Med. 14, 235-41.

Sugishita, Y., Watanabe, M., Fisher, S. A., 2004c. Role of myocardial hypoxia in the remodeling of the embryonic avian cardiac outflow tract. Dev Biol. 267, 294-308.

Sunnassee, A., Shaohua, Z., Liang, R., Liang, L., 2011. Unexpected death of a young woman: is myocardial bridging significant?--A case report and review of literature. Forensic Sci Med Pathol. 7, 42-6.

Takeuchi, S., Imamura, H., Katsumoto, K., Hayashi, I., Katohgi, T., Yozu, R., Ohkura, M., Inoue, T., 1979. New surgical method for repair of anomalous left coronary artery from pulmonary artery. J Thorac Cardiovasc Surg. 78, 7-11.

Tang, N., Wang, L., Esko, J., Giordano, F. J., Huang, Y., Gerber, H. P., Ferrara, N., Johnson, R. S., 2004. Loss of HIF-1alpha in endothelial cells disrupts a hypoxia-driven VEGF autocrine loop necessary for tumorigenesis. Cancer Cell. 6, 485-95.

Taniyama, Y., Morishita, R., Aoki, M., Hiraoka, K., Yamasaki, K., Hashiya, N., Matsumoto, K., Nakamura, T., Kaneda, Y., Ogihara, T., 2002. Angiogenesis and antifibrotic action by hepatocyte growth factor in cardiomyopathy. Hypertension. 40, 47-53.

Taylor, A. J., Rogan, K. M., Virmani, R., 1992. Sudden cardiac death associated with isolated congenital coronary artery anomalies. J Am Coll Cardiol. 20, 640-7.

Tevosian, S. G., Deconinck, A. E., Tanaka, M., Schinke, M., Litovsky, S. H., Izumo, S., Fujiwara, Y., Orkin, S. H., 2000. FOG-2, a cofactor for GATA transcription factors, is essential for heart morphogenesis and development of coronary vessels from epicardium. Cell. 101, 729-39.

Tian, H., Hammer, R. E., Matsumoto, A. M., Russell, D. W., McKnight, S. L., 1998. The hypoxia-responsive transcription factor EPAS1 is essential for catecholamine homeostasis and protection against heart failure during embryonic development. Genes Dev. 12, 3320-4.

Tirziu D, Chorianopoulos E, Moodie KL, Palac RT, Zhuang ZW, Tjwa M, Roncal C, Eriksson U, Fu Q, Elfenbein A, Hall AE, Carmeliet P, Moons L, Simons M, 2007. Myocardial hypertrophy in the absence of external stimuli is induced by angiogenesis in mice. J. Clin. Invest. 117, 3188-3197.

Tomanek, R. J., 2005. Formation of the coronary vasculature during development. Angiogenesis. 8, 273-84.
Tomanek, R. J., 2006. VEGF Family Members Regulate Myocardial Tubulogenesis and Coronary Artery Formation in the Embryo. Circulation Research. 98, 947-953.
Tomanek RJ, Lotun K, Clark EB, Suvarna PR, Hu N, 1998. VEGF and bFGF stimulate myocardial vascularization in embryonic chick. Am. J. Physiol. 274, H1620-H1626.
Tomanek, R. J., Hansen, H. K., Christensen, L. P., 2008. Temporally expressed PDGF and FGF-2 regulate embryonic coronary artery formation and growth. Arterioscler Thromb Vasc Biol. 28, 1237-43.
Tomanek, R. J., Hansen, H. K., Dedkov, E. I., 2006a. Vascular patterning of the quail coronary system during development. Anat Rec A Discov Mol Cell Evol Biol. 288, 989-99.
Tomanek, R. J., Holifield, J. S., Reiter, R. S., Sandra, A., Lin, J. J., 2002. Role of VEGF family members and receptors in coronary vessel formation. Dev Dyn. 225, 233-40.
Tomanek, R. J., Ishii, Y., Holifield, J. S., Sjogren, C. L., Hansen, H. K., Mikawa, T., 2006b. VEGF family members regulate myocardial tubulogenesis and coronary artery formation in the embryo. Circ Res. 98, 947-53.
Tomanek, R. J., Lund, D. D., Yue, X., 2003. Hypoxic induction of myocardial vascularization during development. Adv Exp Med Biol. 543, 139-49.
Tomanek, R. J., Sandra, A., Zheng, W., Brock, T., Bjercke, R. J., Holifield, J. S., 2001. Vascular endothelial growth factor and basic fibroblast growth factor differentially modulate early postnatal coronary angiogenesis. Circ Res. 88, 1135-41.
Vales, L., Kanei, Y., Fox, J., 2010. Coronary artery occlusion and myocardial infarction caused by vasospasm within a myocardial bridge. J Invasive Cardiol. 22, E67-9.
Viragh, S., Challice, C. E., 1981. The origin of the epicardium and the embryonic myocardial circulation in the mouse. Anat Rec. 201, 157-68.
Visconti RP, Richardson CD, Sato TN, 2002. Orchestration of angiogenesis and arteriovenous contribution by angiopoietins and vascular endothelial growth factor (VEGF). Proc. Natl. Acad. Sci. 11, 8219-8224.
Wada, A. M., 2003. Epicardial/Mesothelial Cell Line Retains Vasculogenic Potential of Embryonic Epicardium. Circulation Research. 92, 525-531.
Waldo, K. L., Willner, W., Kirby, M. L., 1990. Origin of the proximal coronary artery stems and a review of ventricular vascularization in the chick embryo. Am J Anat. 188, 109-20.
Ward, N. L., Van Slyke, P., Sturk, C., Cruz, M., Dumont, D. J., 2004. Angiopoietin 1 expression levels in the myocardium direct coronary vessel development. Developmental Dynamics. 229, 500-509.
Waters RE, Rotevatn S, Li P, Annex BH, Yan Z, 2004. Voluntary running induces fiber type-specific angiogenesis in mouse skeletal muscle. Am. J. Physiol. Cell Physiol. 287, C1342–C1348.
Watt, A. J., Battle, M. A., Li, J., Duncan, S. A., 2004. GATA4 is essential for formation of the proepicardium and regulates cardiogenesis. Proc Natl Acad Sci U S A. 101, 12573-8.
Weeks KL, McMullen JR, 2011. The Athlete's Heart vs. the Failing Heart: Can Signaling Explain the Two Distinct Outcomes? Physiology (Bethesda). 26, 97-105.
Wesselhoeft, H., Fawcett, J. S., Johnson, A. L., 1968. Anomalous origin of the left coronary artery from the pulmonary trunk. Its clinical spectrum, pathology, and pathophysiology, based on a review of 140 cases with seven further cases. Circulation. 38, 403-25.

Wessels, A., Perez-Pomares, J. M., 2004. The epicardium and epicardially derived cells (EPDCs) as cardiac stem cells. The Anatomical Record. 276A, 43-57.

Wikenheiser, J., Doughman, Y. Q., Fisher, S. A., Watanabe, M., 2006. Differential levels of tissue hypoxia in the developing chicken heart. Dev Dyn. 235, 115-23.

Wikenheiser, J., Wolfram, J. A., Gargesha, M., Yang, K., Karunamuni, G., Wilson, D. L., Semenza, G. L., Agani, F., Fisher, S. A., Ward, N., Watanabe, M., 2009. Altered hypoxia-inducible factor-1 alpha expression levels correlate with coronary vessel anomalies. Dev Dyn. 238, 2688-700.

Wilm, B., 2005. The serosal mesothelium is a major source of smooth muscle cells of the gut vasculature. Development. 132, 5317-5328.

Witte, M. H., Dellinger, M. T., McDonald, D. M., Nathanson, S. D., Boccardo, F. M., Campisi, C. C., Sleeman, J. P., Gershenwald, J. E., 2011. Lymphangiogenesis and hemangiogenesis: potential targets for therapy. J Surg Oncol. 103, 489-500.

Xu, B., Doughman, Y., Turakhia, M., Jiang, W., Landsettle, C. E., Agani, F. H., Semenza, G. L., Watanabe, M., Yang, Y. C., 2007. Partial rescue of defects in Cited2-deficient embryos by HIF-1alpha heterozygosity. Dev Biol. 301, 130-40.

Yang, K., Doughman, Y. Q., Karunamuni, G., Gu, S., Yang, Y. C., Bader, D. M., Watanabe, M., 2009. Expression of active Notch1 in avian coronary development. Dev Dyn. 238, 162-70.

Yang, Z., Wang, W., Ma, D., Zhang, Y., Wang, L., Xu, S., Chen, B., Miao, D., Cao, K., Ma, W., 2007. Recruitment of stem cells by hepatocyte growth factor via intracoronary gene transfection in the postinfarction heart failure. Sci China C Life Sci. 50, 748-52.

Yang, Z. J., Chen, B., Sheng, Z., Zhang, D. G., Jia, E. Z., Wang, W., Ma, D. C., Zhu, T. B., Wang, L. S., Li, C. J., Wang, H., Cao, K. J., Ma, W. Z., 2010. Improvement of heart function in postinfarct heart failure swine models after hepatocyte growth factor gene transfer: comparison of low-, medium- and high-dose groups. Mol Biol Rep. 37, 2075-81.

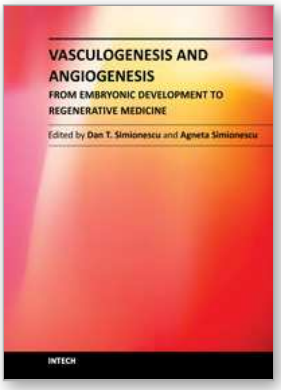
Yin, Z., Haynie, J., Yang, X., Han, B., Kiatchoosakun, S., Restivo, J., Yuan, S., Prabhakar, N. R., Herrup, K., Conlon, R. A., Hoit, B. D., Watanabe, M., Yang, Y. C., 2002. The essential role of Cited2, a negative regulator for HIF-1alpha, in heart development and neurulation. Proc Natl Acad Sci U S A. 99, 10488-93.

Yockman, J. W., Choi, D., Whitten, M. G., Chang, C. W., Kastenmeier, A., Erickson, H., Albanil, A., Lee, M., Kim, S. W., Bull, D. A., 2009. Polymeric gene delivery of ischemia-inducible VEGF significantly attenuates infarct size and apoptosis following myocardial infarct. Gene Ther. 16, 127-35.

Yuniadi, Y., Tai, C. T., Ueng, K. C., Chen, S. A., 2004. Anomaly of the middle cardiac vein? Europace. 6, 451-2.

Zhou, B., Honor, L. B., He, H., Ma, Q., Oh, J. H., Butterfield, C., Lin, R. Z., Melero-Martin, J. M., Dolmatova, E., Duffy, H. S., Gise, A., Zhou, P., Hu, Y. W., Wang, G., Zhang, B., Wang, L., Hall, J. L., Moses, M. A., McGowan, F. X., Pu, W. T., 2011. Adult mouse epicardium modulates myocardial injury by secreting paracrine factors. J Clin Invest. 121, 1894-904.

Zhou, B., Ma, Q., Rajagopal, S., Wu, S. M., Domian, I., Rivera-Feliciano, J., Jiang, D., von Gise, A., Ikeda, S., Chien, K. R., Pu, W. T., 2008. Epicardial progenitors contribute to the cardiomyocyte lineage in the developing heart. Nature. 454, 109-113.



Vasculogenesis and Angiogenesis - from Embryonic Development to Regenerative Medicine

Edited by Dr. Dan Simionescu

ISBN 978-953-307-882-3
Hard cover, 226 pages
Publisher InTech
Published online 07, November, 2011
Published in print edition November, 2011

Vasculogenesis is the process of new blood vessel formation during embryonic development of the cardiovascular system. This is followed by formation of a vascular tree and finally the cardiovascular system with the myriad of blood vessels that nourish all tissues and organs. Angiogenesis, on the other hand is the process by which new blood vessels take shape from existing blood vessels by "sprouting" of endothelial cells thus expanding the vascular tree. Both scenarios are based on activation, migration, proliferation and maturation of unique precursor cells. The study of blood vessel formation is an essential component of embryonic development, congenital malformations, degenerative diseases, inflammation and cancer and thus has widespread appeal to the biomedical field. Moreover, scientists are now harnessing this information for the purpose of building living blood vessel substitutes for replacement of diseased arteries and veins. This book highlights novel advances in the field of vasculogenesis and angiogenesis, including embryogenesis and development, regulation of progenitor cells, cancer and blood vessel regeneration. We consider this book a good initial source of information for graduate students, medical students and scientists interested in the intricacies of blood vessel formation, maturation, disease and replacement.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Michiko Watanabe, Jamie Wikenheiser, Diana Ramirez-Bergeron, Saul Flores, Amir Dangol, Ganga Karunamuni, Akshay Thomas, Monica Montano and Ravi Ashwath (2011). Cardiac Vasculature: Development and Pathology, Vasculogenesis and Angiogenesis - from Embryonic Development to Regenerative Medicine, Dr. Dan Simionescu (Ed.), ISBN: 978-953-307-882-3, InTech, Available from: http://www.intechopen.com/books/vasculogenesis-and-angiogenesis-from-embryonic-development-to-regenerative-medicine/cardiac-vasculature-development-and-pathology

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821